Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/GB05/000623

International filing date: 18 February 2005 (18.02.2005)

Document type: Certified copy of priority document

Document details: Country/Office: GB

Number: 0424080.0

Filing date: 29 October 2004 (29.10.2004)

Date of receipt at the International Bureau: 01 April 2005 (01.04.2005)

Remark: Priority document submitted or transmitted to the International Bureau in

compliance with Rule 17.1(a) or (b)











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2031 OXIDOREDUCTASE

Field of the invention

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The present invention relates to a method of screening for an anti-fungal agent, to fungal 2031 oxidoreductase (2031 OR) enzymes and to diagnosis and therapy of fungal infections.

Background of the invention

Oxidoreductases are a major class of enzymes (EC 1) that catalyse oxidation-reduction (redox) reactions. Redox reactions involve the transfer of reducing equivalents, in the form of electrons or hydrogen atoms, between molecules, i.e., from an electron donor (or reductant) to an electron acceptor (or oxidant). There are many different types of oxidoreductase important for many cellular processes from respiration to protein folding.

The NADH:flavin oxidoreductase /NADH oxidase family of enzymes (InterPro reference IPR001155) contains approximately 263 members mostly of bacterial or yeast origin but with some plant and nematode members. Members of this family use flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as a tightly bound prosthetic group. The flavin prosthetic group can exist in an oxidised (FMN or FAD) or a reduced form (FMNH₂ or FADH₂). These oxidoreductases use the reduced form of nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) as the reductant. A variety of substrates can act as oxidants in the redox reaction.

Old Yellow Enzyme (OYE) is the oldest known member of this family of oxidoreductases (reviewed in Williams and Bruce, 2002, Microbiology 148, 1607-1614). OYE1 (EC 1.6.99.1) was isolated from brewer's bottom yeast by Warburg & Christian (1932, Naturwissenschaften 20, 688) and was the first enzyme for which a cofactor was shown to be required (Theorell, 1935, Biochem. Z. 275, 344-346). This yellow cofactor was found to be riboflavin 5'-phosphate (also known as flavin mononucleotide, FMN). There are 2 OYEs known in Saccharomyces cerevisiae (OYE2 & OYE3) and 2 in Schizosaccharomyces pombe. A great deal is known about

the biochemical mechanism and structure of the enzyme, however, the precise physiological role of the enzyme remains to be elucidated.

OYE has NADPH dehydrogenase activity (see reaction 1 below). The reduced enzyme catalyses the reduction of α/β -unsaturated carbonyl compounds including cyclohexenone (see reaction 2), duroquinone, menadione and N-ethylmaleimide.

- (1) $Enz-FMN + 2NADPH \Leftrightarrow Enz-FMNH_2 + 2NADP^+$
- (2) Enz-FMNH₂ + 2-cyclohexenone \Leftrightarrow Enz-FMN + cyclohexanone



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It has been speculated that OYE may be involved in sterol metabolism (Stott et al, 1993, J. Biol. Chem. 268: 6097-6106) or may be part of the antioxidant defence machinery involved in detoxification of, for example, lipid peroxidation breakdown products (Kohli & Massey, 1998, J. Biol. Chem. 273, 32763-32770). Neither OYE2 nor OYE3 are essential for *S. cerevisiae*. (http://genome-www4.stanford.edu/cgibin/SGD/locus.pl?locus=S0001222;

http://db.yeastgenome.org/cgibin/SGD/locus.pl?locus=YPL171C)

Bacterial members of the NADH:flavin oxidoreductase family include Escherichia coli N-ethylmaleimide reductase, Pseudomonas putida M10 morphinone reductase, Enterobacter cloacae PB2 penterythritol tetranitrate reductase and Azoarcus evansii 2-aminobenzoyl-CoA monooxygenase/reductase (Schühle et al., 2001, J. Bacteriol. 183, 5268-5278).

25 Summary of the invention

The inventors have found a gene for an oxidoreductase of the NADH:flavin oxidoreductase type to be essential for the viability of fungal cells. This finding allows the identification of anti-fungal agents based on their ability to target the oxidoreductase.

The invention provides a new group of oxidoreductases which are herein referred to as 2031 oxidoreductases (2031 ORs) which can be used to screen for anti-fungal

agents. In particular 2031 oxidoreductases from Aspergillus fumigatus, Aspergillus nidulans, Candida albicans, Colletotrichium trifolii, Fusarium graminearum (anamorph Gibberella zeae) Fusarium sporotrichoides, Magnaporthe grisea, Neurospora crassa, Schizosaccharomyces pombe and Ustilago maydis (see Table I) are provided. 2031 OR defines a novel set of oxidoreductases, related to but distinct from OYE and its close relatives, which are essential for the viability of fungal cells.

Accordingly the invention provides the following:

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- a method of identifying an anti-fungal agent which targets an essential protein or gene of a fungus comprising contacting a candidate substance with
 - (i) a NADH: flavin oxidoreductase protein which comprises the sequence shown by SEQ ID NO:3,
 - (ii) a NADH:flavin oxidoreductase protein which is a homologue of (i) and which comprises the sequence shown by SEQ ID NO: 8, 12, 14, 19, 24, 42, 44, 83 or 85,
 - (iii) a protein which has 50% identity with (i) or (ii),
 - (iv) a protein comprising a fragment of (i), (ii) or (iii) which fragment has a length of at least 50 amino acids,
 - (v) a polynucleotide that comprises sequence which encodes (i), (ii), (iii) or (iv),
 - (vi) a polynucleotide comprising sequence which has at least 70% identity with the coding sequence of (v),
 - and determining whether the candidate substance binds or modulates (i), (ii), (iii), (iv), (v) or (vi), wherein binding or modulation of (i), (ii), (iii), (iv), (v) or (vi) indicates that the candidate substance is an anti-fungal agent,
- use of (i), (ii), (iii), (iv), (v) or (vi) as defined above to identify or obtain an antifungal agent,
 - use of an anti-fungal agent identified by the method of the invention in the manufacture of a medicament for prevention or treatment of fungal infection,
- a method of detecting the presence of a fungus in a sample comprising detecting the presence in the said sample of a protein or polynucleotide of the invention,
 - an isolated protein or polynucleotide of the invention,
 - an organism which is transgenic for a polynucleotide of the invention,

- an organism which has been genetically engineered to render a polynucleotide or protein of the invention non-functional or inhibited.

- an antibody which is specific for a protein of the invention,
- a method for preventing or treating a fungal infection comprising administering an anti-fungal agent identified by the screening method of the invention, and
- a fungus which has been killed, or whose growth has been impaired, by inhibition of the expression or activity of a protein or polynucleotide of the invention.

Detailed description of the invention

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As mentioned above the invention relates to use of particular protein and polynucleotide sequences (termed "proteins of the invention" and "polynucleotides of the invention" herein) which are of, or derived from, fungal oxidoreductase proteins and polynucleotides (including homologues and/or fragments of the fungal oxidoreductase proteins and polynucleotides) to identify anti-fungal agents.

As used herein, the term "oxidoreductase" ("OR") may be defined as an enzyme or which is capable of catalysing an oxidation or reduction reaction. The protein of the invention may have an oxidation or reduction activity, such any such activity mentioned herein. The ORs of the invention generally fall within classification EC1 of the enzyme commission.

An essential fungal gene may be defined as one which, when disrupted genetically (for example when not expressed) in a fungus, prevents survival or significantly retards growth of the cell on minimal or defined medium, or in guinnea pigs, mice, rabbits or rats infected with the fungus. In one embodiment the protein of the invention is able to complement such an effect of the genetic disruption. Thus the protein may cause survival (viability) of a fungal cell which does not express its native 2031 oxidoreductase.

A protein or polynucleotide of the invention (or a fungal "2031 OR" gene, nucleic acid or protein) may be defined by similarity in sequence to a another member of the family. As mentioned above this similarity may be based on percentage identity (for example to the sequences shown in the sequence listing).

A protein or polynucleotide of the invention may comprise one or more of the motifs defined by regions 1 - 11 of Figures 1 and 2 (marked at the top of the Figures)

of any of the sequences shown. Thus a protein of the invention may comprise one or more of motifs 1-11 as shown for SEQ ID NO:3 and a polynucleotide of the invention may comprise one or more of motifs 1-11 as shown for SEQ ID NO:1.

Typically the motif is present in substantially the same location as the equivalent location shown in Figure 1 or 2. The equivalent location can be deduced, for example, using any suitable algorithm mentioned herein. In one embodiment the protein or polynucleotide also comprises sequence flanking the motif as shown in Figures 1 or 2 such as sequences of length at least 10, 20 or 30 amino acids/nucleotides flanking the N terminal side and/or C terminal side, or 5' and/or 3' side, of the motif; or sequence which has percentage identity with the flanking sequence.

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The protein of the invention typically comprises at least 2, 3, 5, 8 or 11 of the motifs shown in Figures 1 and 2. The protein preferably comprises at least motif no,6 and/or motif no.9.

The protein or polynucleotide of the invention may align with other 2031 OR polynucleotides or proteins (as shown in SEQ ID Nos. 1-44 and 82-85) showing a greater identity to these than to Old Yellow Enzyme family polynucleotides or proteins

The protein or polynucleotide of the invention typically clusters with other 2031 OR polynucleotides or proteins (as shown in SEQ ID Nos. 1-44 and 82-85) rather than Old Yellow Enzyme family polynucleotides or proteins after phylogenetic analysis, for example with a bootstrap value of greater than 60%.

In one embodiment the protein of the invention has a sequence which matches PFAM profile "oxidored FMN", or INTERPRO profile IPR001155 (for example with an Evalue of e-50 or less) and is closer to a 2031 OR shown in any one of SEQ ID Nos.1-44 and 82-85 than to Old Yellow Enzyme family proteins.

The protein or polynucleotide of the invention may be in isolated form (such as non-cellular form), for example when used in the method of the invention. Preferably, the isolated polynucleotide comprises a 2031 OR gene. Preferably, the isolated protein comprises a 2031 OR. The polynucleotide may comprise native, synthetic or recombinant polynucleotide, and the protein may comprise native, synthetic or recombinant protein. The polynucleotide or protein may comprise combinations of native, synthetic or recombinant polynucleotide or protein, respectively. The polynucleotides and proteins of the invention may have a sequence which is the same

as, or different from, naturally occurring 2031 OR polynucleotides and proteins.

It is to be understood that the term "isolated from" may be read as "of" herein. Therefore references to polynucleotides and proteins being "isolated from" a particular organism include polynucleotides and proteins which were prepared by means other than obtaining them from the organism, such as synthetically or recombinantly.

Preferably, the polynucleotide or protein, is isolated from a fungus, more preferably a filamentous fungus, even more preferably an Ascomycete.

Preferably, the polynucleotide or protein, is isolated from an organism selected from Aspergillus; Blumeria; Candida; Colletotrichium; Cryptococcus; Encephalitozoon; Fusarium; Leptosphaeria; Magnaporthe; Mycosphaerella; Neurospora, Phytophthora; Plasmopara; Pneumocystis; Pyricularia; Pythium; Puccinia; Rhizoctonia; Schizosaccharomyces, Trichophyton; and Ustilago.

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Preferably, the polynucleotide or protein, is isolated from an organism independently selected from a group of genera consisting of Aspergillus, Candida, Colletotrichium, Fusarium, Magnaporthe, Mycosphaerella, Neurospora, Schizosaccharomyces and Ustilago.

Preferably, the polynucleotide or protein, is isolated from an organism selected from the species Aspergillus flavus; Aspergillus fumigatus; Aspergillus nidulans; Aspergillus niger; Aspergillus parasiticus; Aspergillus terreus; Blumeria graminis; Candida albicans; Candida cruzei; Candida glabrata; Candida parapsilosis; Candida tropicalis; Colletotrichium trifolii; Cryptococcus neoformans; Encephalitozoon cuniculi; Fusarium graminarium; Fusarium solani; Fusarium sporotrichoides; Leptosphaeria nodorum; Magnaporthe grisea; Mycosphaerella graminicola; Neurospora crassa; Phytophthora capsici; Phytophthora infestans; Plasmopara viticola; Pneumocystis jiroveci; Puccinia coronata; Puccinia graminis; Pyricularia oryzae; Pythium ultimum; Rhizoctonia solani; Schizzosaccharomyces pombe; Trichophyton interdigitale; Trichophyton rubrum; and Ustilago maydis.

Preferably, the polynucleotide or protein, is isolated from an organism selected from Aspergillus fumigatus; Aspergillus nidulans, Candida albicans, Colletotrichium trifolii, Fusarium graminearum, Fusarium sporotrichoides, Magnaporthe grisea, Mycosphaerella graminicola, Neurospora crassa, Schizosaccharomyces pombe and Ustilago maydis.

The polynucleotide, and preferably the protein, may be isolated from A. fumigatus AF293.

Table I. 2031 OR sequences claimed and their relationship to sequences given in the sequence listing.

	gDNA/EST ^I	Coding	Protein
		sequence(cDNA/mRNA)	
		w/o UTRs ²	
A. fumigatus	SEQ ID No. 1:	SEQ ID No. 2: 115-1384	SEQ ID. No. 3
Oxidoreductase 2031	299-469, 520-1618		
A. fumigatus	SEQ ID No. 4:	SEQ ID No. 5: 1-1266	SEQ ID No. 6
Oxidoreductase 4929	1-180, 267-1352	,	*
A. fumigatus	SEQ ID No. 7:	SEQ ID No. 7: 1-1329	SEQ ID No. 8
Oxidoreductase 1495	1-1329		
A. nidulans 1_112	SEQ ID No. 9:	SEQ ID No. 9:	SEQ ID No. 10
A. manans 1_112	1-1269	1-1269	
C. albicans 2431	SEQ ID No. 11:	SEQ ID No. 11	SEQ ID No. 12
C. Wolcuns 2431	1-1299	1-1299	
C. albicans 2464	SEQ ID No. 13: 1-1110	SEQ ID No. 13: 1-1110	SEQ ID No. 14
C. aibicans 2404	DEQ 10 1101 12 1110		
N. crassa NCU07452.1	SEQ ID No. 15: 1-1305	SEQ ID No. 15: 1-1305	SEQ ID No. 16
	520 25 110. 40. 1 2005		,
N. crassa Oxidoreductase	SEQ ID No. 17: 1-924,1015-	SEQ ID No. 18: 1-1314	SEQ ID No. 19
NCU08900	1362,1435-1476		
M. grisea MG04569.3	SEQ ID No. 20: 1-726, 810-	SEQ ID No. 21: 1-1329	SEQ ID No.22
(pred gene)	1412		,
S. pombe T39956	SEQ ID No. 23: 1-1188	SEQ ID No. 23: 1-1188	SEQ ID No. 24
5. pomoc 155550	DEQ 101.0.23. 1 1100	,	
C. trifolii (EST assembly)	SEQ ID No. 25: 130-777	SEQ ID No. 26: 1-645 (3)	SEQ ID No. 27
C. It your (1551 assembly)	BEQ 15 170, 23, 130 777		
F. sporotrichoides	SEQ ID No. 28: 103-803	SEQ ID No. 29: 1-701	SEQ ID No. 30
FsCon[0063] (ESTs)	222 22 110, 20, 1102 302		
F. sporotrichoides	SEQ ID No. 31: 76-631 (rev	SEQ ID No. 32: 1-556	SEQ ID No.33
FsCon[0237] (ESTs)	comp)	52Q 15 110. 52. 1 550	
	SEQ ID No. 34: 174-657	SEQ ID No. 34: 174-657	SEQ ID No.35
F. sporotrichoides	2EQ 1D 140. 34: 174-037	250 ID 140, 34, 174-037	DEQ ID 110.33
FsCon[0458] (ESTs)			

F. graminearum	SEQ ID No. 36: 1-744	SEQ ID No. 37: 1-742 ⁽⁴⁾	SEQ ID No.38
15771741 (EST)	de .		** W.
F. graminearum	SEQ ID No. 82:	SEQ ID No. 82: 1-1326	SEQ ID No. 83
FG00074.1	1-1326	2005 · Quit	· · ·
M. graminicola mg[0281]	SEQ ID No. 39: 1-647	SEQ ID No. 39: 1-647	SEQ ID No.40
(EST)	. 14		
M. graminicola mga0328f	SEQ ID No. 41: 1-560	SEQ ID No. 41: 1-560	SEQ ID No.42
(EST)	w(·
M. grisea MG03823.3	SEQ ID No. 43: 1-1254	SEQ ID No. 43: 1-1254	SEQ ID No.44
Ustilago maydis	SEQ ID No. 84:	SEQ ID No. 84:	SEQ ID No. 85
Contig 1.2	1-1350	1-1350	

⁽¹⁾ Numbers after SEQ ID Nos. correspond to bases of genomic DNA encoding the protein.

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Bioinformatics analysis was carried out to identify functionally important regions within the fungal 2031 ORs. The 2031 ORs are related to but distinct from the "Old Yellow Enzyme" (OYE) group of yeast enzymes, which also includes ergosterol-binding protein of *Candida albicans*. Comparison of the 2031 ORs with crystal structures of OYE family proteins identified highly conserved residues responsible for the catalytic function of these enzymes. However, the comparisons also identified seven clusters of residues conserved in 2031 enzymes but not OYE enzymes which flanked the substrate binding site and were therefore implicated in determining substrate specificity (regions 2, 4, 6, 7, 8, 10, and 11 in Figures 1 and 2, and Example 4 hereinafter). Four further conserved clusters of residues were identified which, while not predicted to be involved in catalysis, were conserved in 2031 but not OYE and so also distinguish 2031 ORs from OYEs (regions 1, 3, 5, and 9 in Figures 1 and 2, and Example 4 hereinafter).

Variants of the above mentioned polynucleotides and proteins are also provided, and

⁽²⁾RNA sequences are given in the sequence listing with Thymidine (T), although it is understood that *in vivo* Uridine (U) would be present.

^{5 (3)}NA one-base deletion at position 690 of the EST (SEQ ID No. 22) is required to give the best predicted cDNA/protein.

⁽⁴⁾ Two single base deletions are required to optimise translation.

are discussed below.

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In one embodiment, the protein of the invention may comprise an amino acid sequence substantially as set out and independently selected from regions 1 - 11 of any of SEQ ID Nos 3, 6, 8, 10, 12, 14, 16, 19, 22, 24, 27, 30, 33, 35, 38, 40, 42, 44, 83 or 85 as given in Figure 1, or variants thereof. At least one region or motif may be functional.

The polynucleotide of the invention may comprise DNA, such as genomic DNA. The polynucleotide may comprise a sequence substantially as set out and independently selected from regions 1 - 11 of any of SEQ ID Nos. 1, 4, 7, 9, 11, 13, 15, 17, 20, 23, 25, 28, 31, 34, 36, 39 41, 43, 82 or 84 as given in Figure 2, or complements, or variants thereof.

Preferably, the polynucleotide encodes a fungal 2031 OR protein which comprises substantially the amino acid sequences SEQ ID Nos 3, 6, 8, 10, 12, 14, 16, 19, 22, 24, 27, 30, 33, 35, 38, 40, 42, 83 or 85 or a variant thereof.

The polynucleotide may comprise RNA, preferably mRNA, preferably spliced mRNA. Preferably, the polynucleotide comprises substantially the sequence shown as SEQ ID Nos 2, 5, 7, 9, 11, 13, 15, 18, 21, 23, 26, 29, 32, 34, 36, 37, 39, 41, 43, 82 or 84 or a complement, or a variant thereof.

Preferably, the protein comprises substantially the sequences SEQ ID Nos. 3, 6, 8, 10, 12, 14, 16, 19, 22, 24, 27, 30, 33, 35, 38, 40, 42, 44, 83 or 85 or a variant thereof.

Preferably, the protein is encoded by the regions of sequences SEQ ID Nos. 1, 4, 7, 9, 11, 13, 15, 17, 20, 23, 25, 26, 28, 29, 31, 34, 36, 39, 41, 43, 82 or 84 as described in Figure 1. in the column "gDNA/EST" in Table I, or a complement, or a variant thereof.

The polynucleotide may comprise substantially a nucleotide sequence region or motif independently selected from at least one of regions 1-11 from at least one of the sequences SEQ ID Nos. 1, 2, 4, 5, 7, 9, 11, 13, 15, 17, 18, 20, 21, 23, 25, 26, 28, 29, 31, 32, 34, 36, 37, 39, 41, 43, 82 or 84, as given in Figure 2, or a complement, or a variant thereof.

Preferably, the isolated polynucleotide comprises substantially a nucleotide sequence independently selected from the regions and sequences given in the column "gDNA/EST" in Table I.

Preferably, the protein is encoded by a polynucleotide which polynucleotide comprises substantially a sequence independently selected from at least one of the the regions and sequences given in the column "gDNA/EST" in Table I, or a complement or, a variant thereof.

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By the term "native amino acid/polynucleotide/protein", is meant an amino acid, polynucleotide or protein produced naturally from biological sources either *in vivo* or *in vitro*.

By the term "synthetic amino acid/polynucleotide/protein", is meant an amino acid, polynucleotide or protein which has been produced artificially or *de novo* using a DNA or protein synthesis machine known in the art.

By the term "recombinant amino acid/polynucleotide /protein", is meant an amino acid, polynucleotide or protein which has been produced using recombinant DNA or protein technology or methodologies which are known to the skilled technician.

The term "variant", and the terms "substantially the amino acid/polynucleotide/protein sequence" are used herein to refer to related sequences. As discussed below such related sequences are typically homologous to (share percentage identity with) a given sequence, for example over the entire length of the sequence or over a portion of a given length. The related sequence may also be a fragment of the sequence or of a homologous sequence. A variant protein may be encoded by a variant polynucleotide.

By the term "variant", and the terms "substantially the amino acid/polynucleotide/protein sequence", we mean that the sequence has at least 30%, preferably 40%, more preferably 50%, and even more preferably, 60% sequence identity with the amino acid/polynucleotide/protein sequences of any one of the sequences referred to. A sequence which is "substantially the amino acid/polynucleotide/peptide sequence" may be the same as the relevant sequence.

Calculation of percentage identities between different amino acid/polynucleotide/protein sequences may be carried out as follows. A multiple

alignment is first generated by the ClustalX program (pairwise parameters: gap opening 10.0, gap extension 0.1, protein matrix Gonnet 250, DNA matrix IUB; multiple parameters: gap opening 10.0, gap extension 0.2, delay divergent sequences 30%, DNA transition weight 0.5, negative matrix off, protein matrix gonnet series, DNA weight IUB; Protein gap parameters, residue-specific penalties on, hydrophilic penalties on, hydrophilic residues GPSNDQERK, gap separation distance 4, end gap separation off). The percentage identity is then calcluated from the multiple alignment as (N/T)*100, where N is the number of positions at which the two sequences share an identical residue, and T is the total number of positions compared. Alternatively, percentage identity can be calculated as (N/S)*100 where S is the length of the shorter sequence being compared. The amino acid/polynucleotide/protein sequence, or a derivative thereof.

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An amino acid/polynucleotide/protein sequence with a greater identity than 65% of the sequences referred to is also envisaged. An amino acid/polynucleotide/protein sequence with a greater identity than 70% to any of the sequences referred to is also envisaged. An amino acid/polynucleotide/protein sequence with a greater identity than 75% to any of the sequences referred to is also envisaged. An amino acid/polynucleotide/protein sequence with a greater identity than 80% to any of the sequences referred to is also envisaged. Preferably, the amino acid/polynucleotide/protein sequence has 85% identity with any of the sequences referred to, more preferably 90% identity, even more preferably 92% identity, even more preferably 95% identity, even more preferably 97% identity, even more preferably 98% identity and, most preferably, 99% identity with any of the referred to sequences.

The above mentioned percentage identities may be measured over the entire length of the original sequence or over a region of 15, 20, 50 or 100 amino acids/bases of the original sequence. In a preferred embodiment percentage identity is measured with reference to SEQ ID No. 3. Preferably the variant protein has at least 40% identity, such as at least 60% or at least 80% identity with SEQ ID No. 3 or a portion of SEQ ID No. 3.

Alternatively, a substantially similar nucleotide sequence will be encoded by a

sequence which hybridizes to the sequences shown in SEQ ID Nos. 1, 2, 4, 5, 7, 8, 9, 11, 13, 15, 17, 18, 20, 21, 23, 25, 26, 28, 29, 31, 32, 34, 36, 37, 39, 41, 43, 82 or 84 or their complements under stringent conditions. By stringent conditions, we mean the nucleotide hybridises to filter-bound DNA or RNA in 6x sodium chloride/sodium citrate (SSC) at approximately 45°C followed by at least one wash in 0.2x SSC/0.1% SDS at approximately 5-65°C. Alternatively, a substantially similar protein may differ by at least 1, but less than 5, 10, 20, 50 or 100 amino acids from the sequences shown in SEQ ID Nos. 3, 6, 8, 10, 12, 14, 16, 19, 22, 24, 27, 30, 33, 35, 38, 40, 42, 44, 83 or 85. Such differences may each be additions, deletions or substitutions.

Due to the degeneracy of the genetic code, it is clear that any nucleic acid sequence could be varied or changed without substantially affecting the sequence of the protein encoded thereby, to provide a functional variant thereof. Suitable nucleotide variants are those having a sequence altered by the substitution of different codons that encode the same amino acid within the sequence, thus producing a silent change.

Other suitable variants are those having homologous nucleotide sequences but comprising all, or portions of, sequence which are altered by the substitution of different codons that encode an amino acid with a side chain of similar biophysical properties to the amino acid it substitutes, to produce a conservative change. For example small non-polar, hydrophobic amino acids include glycine, alanine, leucine, isoleucine, valine, proline, and methionine. Large non-polar, hydrophobic amino acids include phenylalanine, tryptophan and tyrosine. The polar neutral amino acids include serine, threonine, cysteine, asparagine and glutamine. The positively charged (basic) amino acids include lysine, arginine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Certain organisms, including Candida are known to use non-standard codons compared to those used in the majority of eukaryotes. Any comparisons of polynucleotides and proteins from such organisms with the sequences given here should take these differences into account.

In accurate alignment of protein or DNA sequences the trade-off between optimal matching of sequences and the introduction of gaps to obtain such a match is important. In the case of proteins, the means by which matches are scored is also of significance. The family of PAM matrices (e.g., Dayhoff, M. et al., 1978, Atlas of

protein sequence and structure, Natl. Biomed. Res. Found.) and BLOSUM matrices quantitate the nature and likelihood of conservative substitutions and are used in multiple alignment algorithms, although other, equally applicable matrices will be known to those skilled in the art. The popular multiple alignment program ClustalW, and its windows version ClustalX (Thompson et al., 1994, Nucleic Acids Research, 22, 4673-4680; Thompson et al., 1997, Nucleic Acids Research, 24, 4876-4882) are efficient ways to generate multiple alignments of proteins and DNA.

Use of the Align program is also preferred (Hepperle, D., 2001: Multicolor Sequence Alignment Editor. Institute of Freshwater Ecology and Inland Fisheries, 16775 Stechlin, Germany), although others, such as JalView or Cinema are also suitable.

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Calculation of percentage identities between proteins occurs during the generation of multiple alignments by Clustal. However, these values need to be recalculated if the alignment has been manually improved, or for the deliberate comparison of two sequences. Programs that calculate this value for pairs of protein sequences within an alignment include PROTDIST within the PHYLIP phylogeny package (Felsenstein; http://evolution.gs.washington.edu/ phylip.html) using the "Similarity Table" option as the model for amino acid substitution (P). For DNA/RNA, an identical option exists within the DNADIST program of PHYLIP.

Other modifications in protein sequences are also envisaged and within the scope of the claimed invention, i.e. those which occur during or after translation, e.g. by acetylation, amidation, carboxylation, phosphorylation, proteolytic cleavage or linkage to a ligand.

amino "substantially the "variant", the terms and The term include a fragment of the relevant acid/polynucleotide/protein sequence" also polynucleotide or protein sequences, including a fragment of the homologous sequences (which have percentage identity to a specified sequence) referred to above. polynucleotide fragment will typically comprise at least 10 bases, such as at least 20, 30, 50, 100, 200, 500 or 1000 bases. A protein fragment will typically comprise at least 10 amino acids, such as at least 20, 30, 50, 80, 100, 150, 200, 300, 400 or 500 amino acids. The fragments may lack at least 3 amino acids, such as at least 10, 20 or 30 amino acids of the amino acids from either end of the protein.

The invention provides a method of screening which may be used to identify modulators of 2031 OR proteins or polynucleotides, such as inhibitors of expression or activity of the proteins or polynucleotides of the invention. In one embodiment of the method a candidate substance is contacted with a protein or polynucleotide of the invention and whether or not the candidate substance binds or modulates the protein or polynucleotide is determined.

The modulator may promote (agonise) or inhibit (antagonise) the activity of the protein. A therapeutic modulator (against fungal infection) will inhibit the expression or activity of protein or polynucleotide of the invention.

The method may be carried out *in vitro* (inside or outside a cell) or *in vivo*. In one embodiment the method is carried out on a cell, or cell culture cell extract. The cell may or may not be a cell in which the polynucleotide or protein is naturally present. The cell may or may not be a fungal cell, or may or may not be a cell of any of the fungi mentioned herein. The protein or polynucleotide may be present in a non-cellular form in the method, thus the protein may be in the form of a recombinant protein purified from a cell.

Any suitable binding or activity assay may be used. Methods which determine whether a candidate substance is able to bind the protein or polynucleotide may comprise providing the protein or polynucleotide to a candidate substance and determining whether binding occurs, for example by measuring the amount of the candidate substance which binds the protein or polynucleotide. The binding may be determined by measuring a characteristic of the protein or polynucleotide that changes upon binding, such as spectroscopic changes. The binding may be determined by measuring reaction substrate or product levels in the presence and absence of the candidate and comparing the levels.

The assay format may be a 'band shift' system. This involves determining whether a test candidate advances or retards the protein or polynucleotide on gel electrophoresis relative to the absence of the compound.

The method may be a competitive binding method. This determines whether the candidate is able to inhibit the binding of the protein or polynucleotide to an agent which is known to bind to the protein or polynucleotide, such as an antibody specific for the protein, or a substrate of the protein.

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Whether or not a candidate substance modulates the activity of the protein may be determined by providing the candidate substance to the protein under conditions that permit activity of the protein, and determining whether the candidate substance is able to modulate the activity of the product.

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The activity which is measured may be any of the activities of the protein of the invention mentioned herein, such as oxidoreductase activity. In one embodiment the screening method comprising carrying out a redox reaction in the presence and absence of the candidate substance to determine whether the candidate substance inhibits the oxidoreductase activity of the protein of the invention, wherein the redox reaction is carried out by contacting said protein with NADH or NADPH; and an electron acceptor, under conditions in which in the absence of the candidate substance the protein catalyses reduction of the electron acceptor.

In a preferred embodiment the inhibition of the redox reaction is measured by detecting the amount of NADH or NADPH oxidation, for example by measuring the generation of the oxidised forms of NADH and NADPH spectroscopically. This can be done by measurement at 340nm (see Example 7).

Alternatively, a suitable colourimetric oxidoreductase substrate may be used to measure inhibition, such as methylene blue, phenazine methosulphate or 2, 6-dichlorophenolindophenol.

Suitable candidate substances which can tested in the above methods include antibody products (for example, monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies and CDR-grafted antibodies). Furthermore, combinatorial libraries, defined chemical identities, peptide and peptide mimetics, oligonucleotides and natural product libraries, such as display libraries (e.g. phage display libraries) may also be tested. The candidate substances may be chemical compounds. Batches of the candidate substances may be used in an initial screen of, for example, ten substances per reaction, and the substances from batches which show inhibition tested individually.

According to a further aspect of the present invention, there is provided a polynucleotide or protein of the invention for use as a medicament or in diagnosis.

The polynucleotide or protein may be modified prior to use, preferably to produce a derivative or variant thereof. The polynucleotide or protein may be

derivatised. The protein may be modified by epitope tagging, addition of fusion partners or purification tags such as glutathione S-transferase, multiple histidines or maltose binding protein, addition of green fluorescent protein, covalent attachment of molecules including biotin or fluorescent tags, incorporation of selenomethionine, inclusion or attachment of radioisotopes or fluorescent/non-fluorescent lanthanide chelates. The polynucleotide may be modified by methylation or attachment of digoxygenin (DIG) or by addition of sequence encoding the above tags, proteins or epitopes.

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Preferably, the medicament is adapted to retard or prevent a fungal infection. The fungal infection may be in human, animal or plant. The polynucleotide or protein may be used for the development of a drug. The polynucleotide or protein may be used in, or for the generation of, a molecular model of said polynucleotide or said protein.

According to a further aspect of the present invention, there is provided use of a polynucleotide or protein of the invention for the preparation of a medicament for the treatment of a fungal infection.

The polynucleotide or protein may be modified prior to use, preferably to produce a derivative or variant thereof. The polynucleotide or protein may be derivatised. The polynucleotide or protein may not be modified or derivatised.

Preferably, the medicament is adapted to retard or prevent a fungal infection. The treatment may comprise retarding or preventing fungal infection. Preferably, the drug and/or medicament comprises an inhibitor, preferably a 2031 OR inhibitor. Preferably, the drug or medicament is adapted to inhibit expression and/or activity of the polynucleotide or a fragment thereof, and/or the function of the protein or a fragment thereof.

Preferably, the fungal infection comprises an infection by a fungus, more preferably an Ascomycete, and even more preferably, an organism selected from the genera Aspergillus; Blumeria; Candida; Colletotrichium; Cryptococcus; Encephalitozoon; Fusarium; Leptosphaeria; Magnaporthe; Mycosphaerella; Neurospora, Phytophthora; Plasmopara; Pneumocystis; Pyricularia; Pythium; Puccinia; Rhizoctonia; Schizosaccharomyces, Trichophyton; and Ustilago.

Preferably, the fungal infection comprises an infection by an organism selected from the genera Aspergillus, Candida, Colletotrichium, Fusarium, Magnaporthe,

Mycosphaerella and Ustilago.

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Preferably, the fungal infection comprises an infection by an organism selected from the species Aspergillus flavus; Aspergillus fumigatus; Aspergillus nidulans; Aspergillus niger; Aspergillus parasiticus; Aspergillus terreus; Blumeria graminis; Candida albicans; Candida cruzei; Candida glabrata; Candida parapsilosis; Candida tropicalis; Colletotrichium trifolii; Cryptococcus neoformans; Encephalitozoon cuniculi; Fusarium graminarium; Fusarium solani; Fusarium sporotrichoides; Leptosphaeria nodorum; Magnaporthe grisea; Mycosphaerella graminicola; Phytophthora capsici; Phytophthora infestans; Plasmopara viticola; Pneumocystis jiroveci; Puccinia coronata; Puccinia graminis; Pyricularia oryzae; Pythium ultimum; Rhizoctonia solani; Trichophyton interdigitale; Trichophyton rubrum; and Ustilago maydis.

Preferably, the fungal infection comprises an infection by an organism selected from the species Aspergillus fumigatus; Aspergillus nidulans, Candida albicans, Colletotrichium trifolii, Fusarium graminearum, Fusarium sporotrichoides, Magnaporthe grisea, Mycosphaerella graminicola and Ustilago maydis.

According to another aspect of the present invention, there is provided a method of detecting the presence of a fungal infection in an individual, said method comprising:-

- 20 (i) obtaining a sample from an organism; and
 - (ii) detecting in the said sample the presence of a polynucleotide or protein of the invention.

The individual may be a person (human) or animal (such as a mammal or bird) or a plant. The fungal infection may arise from infection with an organism selected from the genera Aspergillus; Blumeria; Candida; Colletotrichium; Cryptococcus; Encephalitozoon; Fusarium; Leptosphaeria; Magnaporthe; Mycosphaerella; Phytophthora; Plasmopara; Pneumocystis; Pyricularia; Pythium; Puccinia; Rhizoctonia; Trichophyton; and Ustilago

The fungal infection may arise from infection with an organism selected from the species Aspergillus flavus; Aspergillus fumigatus; Aspergillus nidulans; Aspergillus niger; Aspergillus parasiticus; Aspergillus terreus; Blumeria graminis; Candida albicans; Candida cruzei; Candida glabrata; Candida parapsilosis; Candida

tropicalis; Colletotrichium trifolii; Cryptococcus neoformans; Encephalitozoon cuniculi; Fusarium graminarium; Fusarium solani; Fusarium sporotrichoides; Leptosphaeria nodorum; Magnaporthe grisea; Mycosphaerella graminicola; Phytophthora capsici; Phytophthora infestans; Plasmopara viticola; Pneumocystis jiroveci; Puccinia coronata; Puccinia graminis; Pyricularia oryzae; Pythium ultimum; Rhizoctonia solani; Trichophyton interdigitale; Trichophyton rubrum; and Ustilago maydis.

Preferably, the sample comprises a biological sample which, preferably, comprises nucleic acid and/or protein. In one embodiment of the method the nucleic acid or protein is purified (at least partially) from the sample before the detection is performed.

Where the organism is Aspergillus fumigatus, Aspergillus nidulans or Aspergillus niger, the sample may comprise sputum, bronchoalveloar lavage, urine, respiratory specimens, endotracheal aspirates, sterile specimens obtained by an invasive procedure such as vitreous tap, tympanocentesis, brain biopsy or aspiration, nasal or sinus specimens, blood, tissue or autopsy.

Where the organism is Magnaporthe grisea the sample may comprise rice leaf or rice stem.

Preferably, said detecting of the presence in the said sample of a polynucleotide as defined by the first or third aspect comprises use of at least one oligonucleotide pair adapted to be used for amplification of DNA, preferably genomic, more preferably, fungal genomic DNA. The amplification may be PCR amplification.

Preferably, the PCR amplification employs at least one primer pair comprising a polynucleotide selected from the group consisting of:

Aspergillus fumigatus; SEQ ID Nos 67 and 68 for SEQ ID No. 1; SEQ ID Nos 69 and 70 for SEQ ID No. 4; and SEQ ID Nos 71 and 72 for SEQ ID No. 7.

Candida albicans; SEQ ID Nos 73 and 74 for SEQ ID No. 11.

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Magnaporthe grisea; SEQ ID Nos 75 and 76 for SEQ ID No. 20.

Preferably, said detecting comprises subjecting the amplified DNA to size analysis, preferably, electrophoresis and, preferably, comparing the results to a positive control and, preferably, a negative control. Said detecting may also comprise sequencing of the amplified DNA to demonstrate the correct sequence.

Preferably, said detecting of the presence in the said sample of a protein comprises use of a monoclonal or polyclonal antibody directed to part or all of the protein of the invention.

According to a further aspect of the present invention, there is provided a recombinant DNA molecule or vector comprising a polynucleotide of the invention.

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The recombinant DNA molecule or vector may comprise an expression cassette. Preferably, the recombinant DNA molecule or vector comprises an expression vector. Preferably, the polynucleotide sequence is operatively linked to an expression control sequence. A suitable control sequence may comprise a promoter, an enhancer etc.

According to another aspect of the present invention, there is provided a cell containing a polynucleotide, recombinant DNA molecule or vector of the invention.

The cell may be transformed or transfected with the polynucleotide, recombinant DNA molecule or vector by suitable means. Preferably, the cell produces a recombinant protein of the invention.

The invention also provides an organism which is transgenic for the polynucleotide of the invention (whose cells may be the same as the cells of the invention mentioned herein). Such an organism is typically a fungus, such as any genera or species of fungus mentioned herein. The organism may be microorganism, such as a bacterium, virus or yeast. The organism may be a plant, animal (including birds and mammals), such as any of the animals mentioned herein.

The organism may be produced by introduction of the polynucleotide of the invention into a cell of the organism, and in the case of a multicellular organism allowing the cell to grow into a whole organism.

According to a further aspect of the present invention, there is provided a cell in which a native polynucleotide or protein of the invention protein is non-functional and/or inhibited. The cell may be of, or present in, a multicellular organism.

The cell may be a mutant cell. The cell is typically a fungal cell, such as of any genera or species of fungus mentioned herein. A preferred means of generating the cell is to modify the polynucleotide of the invention, such that the polynucleotide is non-functional. This modification may be to cause a mutation, which disrupts the expression or function of a gene product. Such mutations may be to the nucleic acid sequences that act as 5' or 3' regulatory sequences for the polynucleotide, or may be a mutation introduced into

the coding sequence of the polynucleotide. Functional deletion of the polynucleotide may be, for example, by mutation of the polynucleotide in the form of nucleotide substitution, addition or, preferably, nucleotide deletion.

The polynucleotide may be made non-functional and/or inhibited by:

- (i) shifting the reading frame of the coding sequence of the polynucleotide;
 - (ii) adding, substituting or deleting amino acids in the protein encoded by the polynucleotide; or
 - (iii) partially or entirely deleting the DNA coding for the polynucleotide and/or the upstream and downstream regulatory sequences associated with the polynucleotide.
- 10 (iv) inserting DNA into the coding or non-coding regions.

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A preferred means of introducing a mutation into a polynucleotide is to utilize molecular biology techniques specifically to target the polynucleotide which is to be mutated. Mutations may be induced using a DNA molecule. A most preferred means of introducing a mutation is to use a DNA molecule that has been especially prepared such that homologous recombination occurs between the target polynucleotide and the DNA molecule. When this is the case, the DNA molecule, which may be double stranded, may contain base sequences similar or identical to the target polynucleotide to allow the DNA molecule to hybridize to (and subsequently recombine with) the target.

It is also possible to provide a cell in which the polynucleotide is non-functional and/or inhibited without introducing a mutation into the gene or its regulatory regions. This may be done by using specific inhibitors. Examples of such inhibitors include agents that prevent transcription of the polynucleotide, or prevent translation, expression or disrupt post-translational modification. Alternatively, the inhibitor may be an agent that increases degradation of the gene product (e.g. a specific proteolytic enzyme). Equally, the inhibitor may be an agent which prevents the polynucleotide product from functioning, such as neutralizing antibodies (for instance an anti-2031 OR antibody). The inhibitor may also be an antisense oligonucleotide, or any synthetic chemical capable of inhibiting expression of the gene or the stability and/or function of the protein. The inhibitor may also be a protein which interacts with the 2031 OR to prevent its function. The inhibitor may also be an RNA molecule which causes inhibition by RNA interference. In one

embodiment the antisense polynucleotide or RNA molecule which causes RNA interference are examples of polynucleotides of the invention.

According to a further aspect, there is provided an antibody exhibiting immunospecificity for a protein of the invention. The antibody may be used as a diagnostic reagent.

The antibody may be monoclonal or polyclonal, and may be raised in mouse, rat, rabbit, chicken, turkey, horse, goat or donkey. The antibody may be raised against one or all of the proteins together, or may be raised against proteolytic or recombinant fragments.

For the purposes of this invention, the term "antibody", unless specified to the contrary, includes fragments which bind a protein of the invention. Such fragments include Fv, F(ab') and F(ab')₂ fragments, as well as single chain antibodies. Furthermore, the antibodies and fragment thereof may be chimeric antibodies, CDR-grafted antibodies or humanised antibodies.

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Administration

The formulation of any of the therapeutic substances (e.g. proteins, polynucleotides or modulators) mentioned herein will depend upon factors such as the nature of the substance and the condition to be treated. Any such substance may be administered in a variety of dosage forms. It may be administered orally (e.g. as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules), parenterally, subcutaneously, intravenously, intramuscularly, intrasternally, transdermally or by infusion techniques. The substance may also be administered as suppositories. A physician will be able to determine the required route of administration for each particular patient.

Typically the substance is formulated for use with a pharmaceutically acceptable carrier or diluent. The pharmaceutical carrier or diluent may be, for example, an isotonic solution. For example, solid oral forms may contain, together with the active compound, diluents, e.g. lactose, dextrose, saccharose, cellulose, corn starch or potato starch; lubricants, e.g. silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; binding agents; e.g. starches, arabic gums, gelatin, methylcellulose, carboxymethylcellulose or polyvinyl pyrrolidone; disaggregating

agents, e.g. starch, alginic acid, alginates or sodium starch glycolate; effervescing mixtures; dyestuffs; sweeteners; wetting agents, such as lecithin, polysorbates, laurylsulphates; and, in general, non-toxic and pharmacologically inactive substances used in pharmaceutical formulations. Such pharmaceutical preparations may be manufactured in known manner, for example, by means of mixing, granulating, tabletting, sugar-coating, or film coating processes.

Liquid dispersions for oral administration may be syrups, emulsions and suspensions. The syrups may contain as carriers, for example, saccharose or saccharose with glycerine and/or mannitol and/or sorbitol. Suspensions and emulsions may contain as carrier, for example a natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose, or polyvinyl alcohol. The suspensions or solutions for intramuscular injections may contain, together with the active compound, a pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride.

Solutions for intravenous or infusions may contain as carrier, for example, sterile water or preferably they may be in the form of sterile, aqueous, isotonic saline solutions.

A therapeutically effective non-toxic amount of substance is administered. The dose may be determined according to various parameters, especially according to the substance used; the age, weight and condition of the patient to be treated; the route of administration; and the required regimen. Again, a physician will be able to determine the required route of administration and dosage for any particular patient. A typical daily dose is from about 0.1 to 50 mg per kg, preferably from about 0.1mg/kg to 10mg/kg of body weight, according to the activity of the specific inhibitor, the age, weight and conditions of the subject to be treated, the type and severity of the disease and the frequency and route of administration. Preferably, daily dosage levels are from 5 mg to 2 g.

Agricultural use

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Modulators identified by the method of the invention may be administered to plants in order to prevent or treat fungal infections. The modulators are normally applied in the form of compositions together with one or more agriculturally acceptable carriers or

diluents and can be applied to the crop area or plant to be treated, simultaneously or in succession with further compounds.

The modulators of the invention can be applied together with carriers, surfactants or application-promoting adjuvants customarily employed in the art of formulation. Suitable carriers and diluents correspond to substances ordinarily employed in formulation technology, e.g. natural or regenerated mineral substances, solvents, dispersants, wetting agents, tackifiers, binders or fertilizers.

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A preferred method of applying the modulators of the present invention or an agrochemical composition which contains them is leaf application. The number of applications and the rate of application depend on the intensity of infection by the fungus. However, the active ingredients can also penetrate the plant through the roots via the soil (systemic action) by impregnating the locus of the plant with a liquid composition, or by applying the compounds in solid form to the soil, e.g. in granular form (soil application). The active ingredients may also be applied to seeds (coating) by impregnating the seeds either with a liquid formulation containing active ingredients, or coating them with a solid formulation. In special cases, further types of application are also possible, for example, selective treatment of the plant stems or buds.

The active ingredients are used in unmodified form or, preferably, together with the adjuvants conventionally employed in the art of formulation, and are therefore formulated in known manner to emulsifiable concentrates, coatable pastes, directly sprayable or dilutable solutions, dilute emulsions, wettable powders, soluble powders, dusts, granulates, and also encapsulations, for example, in polymer substances. Like the nature of the compositions, the methods of application, such as spraying, atomizing, dusting, scattering or pouring, are chosen in accordance with the intended objectives and the prevailing circumstances. Advantageous rates of application are normally from 50g to 5kg of active ingredient (a.i.) per hectare ("ha", approximately 2.471 acres), preferably from 100g to 2kg a.i./ha, most preferably from 200g to 500g a.i./ha.

The formulations, compositions or preparations containing the active ingredients and, where appropriate, a solid or liquid adjuvant, are prepared in known manner, for example by homogeneously mixing and/or grinding active ingredients with extenders,

for example solvents, solid carriers and, where appropriate, surface-active compounds (surfactants).

Suitable solvents include aromatic hydrocarbons, preferably the fractions having 8 to 12 carbon atoms, for example, xylene mixtures or substituted naphthalenes, phthalates such as dibutyl phthalate or dioctyl phthalate, aliphatic hydrocarbons such as cyclohexane or paraffins, alcohols and glycols and their ethers and esters, such as ethanol, ethylene glycol, monomethyl or monoethyl ether, ketones such as cyclohexanone, strongly polar solvents such as N-methyl-2-pyrrolidone, dimethyl sulfoxide or dimethyl formamide, as well as epoxidized vegetable oils such as epoxidized coconut oil or soybean oil; or water.

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The solid carriers used e.g. for dusts and dispersible powders, are normally natural mineral fillers such as calcite, talcum, kaolin, montmorillonite or attapulgite. In order to improve the physical properties it is also possible to add highly dispersed silicic acid or highly dispersed absorbent polymers. Suitable granulated adsorptive carriers are porous types, for example pumice, broken brick, sepiolite or bentonite; and suitable nonsorbent carriers are materials such as calcite or sand. In addition, a great number of pregranulated materials of inorganic or organic nature can be used, e.g. especially dolomite or pulverized plant residues.

Depending on the nature of the active ingredient to be used in the formulation, suitable surface-active compounds are nonionic, cationic and/or anionic surfactants having good emulsifying, dispersing and wetting properties. The term "surfactants" will also be understood as comprising mixtures of surfactants.

Suitable anionic surfactants can be both water-soluble soaps and water-soluble synthetic surface-active compounds. Suitable soaps are the alkali metal salts, alkaline earth metal salts or unsubstituted or substituted ammonium salts of higher fatty acids (chains of 10 to 22 carbon atoms), for example the sodium or potassium salts of oleic or stearic acid, or of natural fatty acid mixtures which can be obtained for example from coconut oil or tallow oil. The fatty acid methyltaurin salts may also be used.

More frequently, however, so-called synthetic surfactants are used, especially fatty sulfonates, fatty sulfates, sulfonated benzimidazole derivatives or alkylarylsulfonates. The fatty sulfonates or sulfates are usually in the form of alkali metal salts, alkaline earth metal salts or unsubstituted or substituted ammoniums salts

and have a 8 to 22 carbon alkyl radical which also includes the alkyl moiety of alkyl radicals, for example, the sodium or calcium salt of lignonsulfonic acid, of dodecylsulfate or of a mixture of fatty alcohol sulfates obtained from natural fatty acids. These compounds also comprise the salts of sulfuric acid esters and sulfonic acids of fatty alcohol/ethylene oxide adducts. The sulfonated benzimidazole derivatives preferably contain 2 sulfonic acid groups and one fatty acid radical containing 8 to 22 carbon atoms. Examples of alkylarylsulfonates are the sodium, calcium triethanolamine of or salts dodecylbenzenesulfonic dibutylnaphthalenesulfonic acid, or of a naphthalenesulfonic acid/formaldehyde condensation product. Also suitable are corresponding phosphates, e.g. salts of the phosphoric acid ester of an adduct of p-nonylphenol with 4 to 14 moles of ethylene oxide.

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Non-ionic surfactants are preferably polyglycol ether derivatives of aliphatic or cycloaliphatic alcohols, or saturated or unsaturated fatty acids and alkylphenols, said derivatives containing 3 to 30 glycol ether groups and 8 to 20 carbon atoms in the (aliphatic) hydrocarbon moiety and 6 to 18 carbon atoms in the alkyl moiety of the alkylphenols.

Further suitable non-ionic surfactants are the water-soluble adducts of polyethylene oxide with polypropylene glycol, ethylenediamine propylene glycol and alkylpolypropylene glycol containing 1 to 10 carbon atoms in the alkyl chain, which adducts contain 20 to 250 ethylene glycol ether groups and 10 to 100 propylene glycol ether groups. These compounds usually contain 1 to 5 ethylene glycol units per propylene glycol unit.

Representative examples of non-ionic surfactants are nonylphenolpolyethoxyethanols, castor oil polyglycol ethers, polypropylene/polyethylene oxide adducts, tributylphenoxypolyethoxyethanol, polyethylene glycol and octylphenoxyethoxyethanol. Fatty acid esters of polyoxyethylene sorbitan and polyoxyethylene sorbitan trioleate are also suitable nonionic surfactants.

Cationic surfactants are preferably quaternary ammonium salts which have, as N-substituent, at least one C_8 - C_{22} alkyl radical and, as further substituents, lower unsubstituted or halogenated alkyl, benzyl or lower hydroxyalkyl radicals. The salts

are preferably in the form of halides, methylsulfates or ethylsulfates, e.g. stearyltrimethylammonium chloride or benzyldi(2-chloroethyl)ethylammonium bromide.

The surfactants customarily employed in the art of formulation are described, for example, in "McCutcheon's Detergents and Emulsifiers Annual", MC Publishing Corp. Ringwood, New Jersey, 1979, and Sisely and Wood, "Encyclopaedia of Surface Active Agents," Chemical Publishing Co., Inc. New York, 1980.

The agrochemical compositions usually contain from about 0.1 to about 99% preferably about 0.1 to about 95%, and most preferably from about 3 to about 90% of the active ingredient, from about 1 to about 99.9%, preferably from about 1 to 99%, and most preferably from about 5 to about 95% of a solid or liquid adjuvant, and from about 0 to about 25%, preferably about 0.1 to about 25%, and most preferably from about 0.1 to about 20% of a surfactant. Whereas commercial products are preferably formulated as concentrates, the end user will normally employ dilute formulations.

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All of the features described herein may be combined with any of the above aspects, in any combination.

Embodiments of the invention will now be described by way of example, with reference to the accompanying drawings in which:-

Figure 1 illustrates a multiple sequence alignment of amino acid sequences corresponding to fungal and bacterial 2031 and OYE family oxidoreductases;

Figure 2 illustrates a multiple sequence alignment of nucleic acid sequences corresponding to fungal 2031 and family oxidoreductases;

Figure 3A illustrates the expression of recombinant 2031 OR; B shows purified recombinant 2031 OR.

Figure 4. Phylogenetic tree showing relationships between A. fumigatus 2031 OR and similar proteins. This demonstrates a 2031 OR clade, which can be distinguished from the OYE proteins;

Figure 5 illustrates reduction of a range of substrates by recombinant 2031 OR.

Figure 6 illustrates the inhibition of 2031 OR by two compounds identified from a screen.

EXAMPLES

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Example 1. Identification of an essential gene in Aspergillus fumigatus

An essential region of the A. fumigatus genome was identified using the mycobank technology as described in patent WO00177295A1 with the following modifications:

Re-haploidisation (section 1.6):

P24 lines 11-18: Conidia (A. fumigatus) were collected from a stable diploid transformant colony and approximately $3x10^4$ spores were used to inoculate 1 ml of SAB broth containing 1mg/ml FPA. This culture was incubated with shaking (200 rpm) at 37°C for 20 hours. 100µl of the culture was spread onto complete media containing 0.2 mg/ml FPA and incubated at 37 °C for 3 days or until rapidly growing sectors emerged. Conidia were collected from each sector and plated onto nitrate, nitrite and hypoxanthine media and the nitrogen utilisation profiles of the resulting conidia assessed. Colonies with the nitrogen utilisation profiles of the parental strains indicated breakdown of the diploid to a haploid. 44 haploid sectors were isolated from transformant 2031. None of the haploids isolated were hygromycin resistant indicating the insertion of the hph gene into a portion of the genome required for function.

Transformation (section 1.7):

P25 line 9: Plasmid pAN7-1 linearised with HindIII was used as the transforming vector. PAN7-1 carries the *hph* gene which confers hygromycin resistance.

- P25 lines 17-20: 1 ml of cold YED was added to the cuvette and incubated at 37 °C for 1 h. Aliquots were spread on selective agar (complete media with 250 μg/ml hygromycin). Colonies growing on selective media were deemed putative transformants.
- The point of insertion was identified using the plasmid rescue method outlined on page 31 lines 5-17. The insertion site was confirmed by employing PCR: Using the sequence obtained from plasmid rescue data a primer was designed within the

sequence of pAN7-1 and a complementary primer was designed within the predicted sequence near the point of insertion. Genomic DNA isolated from the diploid 2031 was used as a template.

The resulting DNA sequence (experiment 2031, with 175 bases of upstream pAN7.1 sequence removed) corresponds to the gDNA sequence immediately downstream of the insertion site and is given as SEQ ID No. 45.

Example 2. Characterisation of the essential gene

2.1 Genome analysis

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The TIGR A. fumigatus database (www.TIGR.org) was searched (blastn) with the sequence SEQ ID No. 45, identified in Example 1 above, and a match to contig 4798 (Eval 4.6e-148) was identified. The appropriate region of the contig sequence was down-loaded from www.tigr.org and gene predictions carried out using Genscan (genes.mit.edu/GENSCAN.html; Settings; organism = vertebrate; Suboptimal exon cutoff = 1.00).

The *ab initio* prediction of genes from genomes is known to be an inaccurate process (Burset, M. and Guigó, 1996, Genomics, 34, 353-367) and this is particularly so when the programs used have not been specifically trained for the genome under examination (as is the case here). It is therefore necessary to carefully examine the predictions, to compare any predicted genes with any homologous proteins, and to exploit the operative's knowledge of fungal gene structure, and thus to arrive at an informed prediction. The predicted genes were therefore compared with similar sequences using blastp (http:// blast.genome.ad.jp/), the multiple alignment program ClustalX (Thompson et al., 1997, Nucleic Acids Research, 24:4876-4882), and the alignment editor/ viewer Align (Hepperle, D., 2001: Multicolor Sequence Alignment Editor. Institute of Freshwater Ecology and Inland Fisheries, 16775 Stechlin, Germany). Gene structures were visualised and modified using Artemis (http://www.sanger.ac.uk/Software/Artemis/; Rutherford et al., 2000, Bioinformatics 16, 944-945).

The gene adjacent to the insertion site corresponded to bases 299-469 (exon 1) and bases 520-1618 (exon 2) of the genomic sequence given as SEQ ID No. 1. The protein sequence for the gene is given as SEQ ID No. 3. The insertion site was 735 bases upstream of the 5' ATG start of the gene.

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Searches of the protein databases at http://blast.genome.ad.jp/ showed that protein SEQ ID No. 3 is a member of the NADH-dependent flavin oxidoreductase family. This protein is henceforth referred to as 2031 oxidoreductase (2031 OR; having come from mycobank experiment 2031). Other 2031 OR-like proteins were also identified (see Example 4.1). The NADH-dependent flavin oxidoreductase family also includes Old Yellow Enzyme (OYE), from *S. cerevisiae* and other fungi, although 2031 ORs can be distinguished from OYEs.

Referring to Figures 1, there is shown a multiple alignment of the 2031 OR amino acid sequence from A. fumigatus along with related ORs from other fungi and bacteria (see also Example 4). Regions 1-11 refer to amino acids conserved between ORs.

Fungal 2031 ORs are given by: SEQ ID Nos. 3, 6 and 8, A. fumigatus; SEQ ID No. 10, A.nidulans; SEQ ID Nos. 12 and 14, C. albicans; SEQ ID Nos. 16 and 19, N. crassa; SEQ ID Nos 22 and 44, M. grisea; SEQ ID No. 24, (NP_595868), S. pombe; SEQ ID No. 27, C. trifolii; SEQ ID Nos. 30, 33 and 35, F. sporotrichioides; SEQ ID Nos. 38 and 83, F. graminearumSEQ ID Nos. 40 and 42, M. graminicola; SEQ ID No. 85, U. maydis.

Bacterial ORs resembling 2031 are: T44612 (*Pseudomonas putida*), SEQ ID No. 86; NP_625402 (*Streptomyces coelicolor*), SEQ ID No. 87; NP_295913 (*Deinococcus radiodurans*), SEQ ID No. 88; AF320254 (*Azoarcus evansii*, SEQ ID No. 89.

Fungal ORs similar to the Old Yellow Enzyme family (originally identified in *S. cerevisiae*): *A. fumigatus*, Af4875 and Af4961, SEQ ID Nos. 90 and 91 respectively; *C. albicans*, Ca2460 and A36990, SEQ ID Nos. 92 and 93 respectively; *N. crassa*, Nc4452, SEQ ID No. 94; *S. cerevisiae*, OYE1, OYE2 and OYE3, SEQ ID Nos. 95-97 respectively.

Details of the sequence searches that identified the ORs other than SEQ ID No. 3, and methods for the construction of multiple alignments are given in Example 4 hereinafter.

Referring to Figure 2, there is shown a multiple alignment of the nucleotide sequence of 2031 OR from A. fumigatus along with related 2031 ORs from other fungi and bacteria (see also Example 4). Regions 1-11 refer to amino acids conserved between 2031 ORs at the amino acid level. Fungal 2031 ORs are given by SEQ ID No.: SEQ ID Nos. 1, 2, 4, 5, and 7, A. fumigatus; SEQ ID No. 9, A.nidulans; SEQ ID Nos. 11 and 13, C. albicans; SEQ ID Nos. 15, 17 and 18, N. crassa; SEQ ID Nos. 20, 21 and 43, M. grisea; SEQ ID No. 23 (NP_595868), S. pombe; SEQ ID Nos. 25 and 26, C. trifolii; SEQ ID Nos. 28, 29, 31, 32 and 34, F. sporotrichioides; SEQ ID Nos. 36, 37 and 82, F. graminearum; SEQ ID Nos. 39 and 41, M. graminicola; SEQ ID No. 84, U. maydis.

Details of the sequence searches that identified the ORs, and methods for the construction of multiple alignments are given in Example 4 hereinafter.

2.2 Genomic Sequencing of Genes

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Following the above bioinformatic analyses, the genomic sequences of 2031 OR was experimentally determined.

2.2.1 Bacterial and Fungal Strains

For bacterial cloning, *E. coli* strains Top10 (Invitrogen) and select96 (Promega) were used in accordance with manufacturers' instructions.

A. fumigatus clinical isolate AF293 (ref. No. NCPF7367; available to the public from the NCPF repository; Bristol, U.K.); the CBS repository (Belgium) or from Dr. David Denning's clinical isolate culture collection, Hope Hospital, Salford. U.K.) is the preferred strain according to the present invention. AF293 was isolated in 1993 from the lung biopsy of a patient with invasive aspergillosis and aplastic anaemia. It was donated by Shrewsbury PHLS.

2.2.2 Purification of A. fumigatus genomic DNA

To obtain mycelial material for genomic DNA isolation, approximately 10⁷ A. fumigatus conidia were inoculated in 50 ml of Vogel's minimal medium and incubated with shaking at 200 rpm until late exponential phase (18-24 h) at 37°C. Mycelium was

dried down onto Whatmann 54 paper using a Buckner funnel and a side-arm flask attached to a vacuum pump and washed with PBS/Tween. At this point, the mycelium could be freeze-dried for extraction at a later date.

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The mycelium (fresh or freeze dried) was ground to a powder using liquid nitrogen in a -20°C cooled mortar. The ground biomass was transferred to 50 ml tubes on ice up to the 10 ml mark. An equal volume of extraction buffer (0.7 M NaCl; 0.1 M Na₂SO₃; 0.1 M Tris-HCl pH 7.5; 0.05 M EDTA; 1%(w/v) SDS; pre-warmed to 65°C) was then added to each tube, mixed thoroughly with a pipette tip and incubated at 65°C for 20 minutes in a water bath. A volume of chloroform/isoamyl alcohol (24:1) equivalent to the volume of the original biomass was then added to each tube, tubes were mixed thoroughly and incubated on ice for 30 min. Tubes were then centrifuged at 3,500 x g for 30 min and the aqueous phase carefully transferred to fresh 50 ml tubes without disturbing the interface.

An equal volume of chloroform/isoamyl alcohol (24:1) was added, the tubes vortexed and incubated on ice for 15 minutes. Tubes were then spun at 3,500 x g for 15 minutes. After this spin, if large amounts of precipitate were still present, the supernatant was removed and the chloroform:isoamyl alcohol step repeated. The supernatant was removed and placed in clean sterile Oak Ridge tubes. An equal volume of isopropanol was added and mixed gently. Tubes were incubated at room temperature for at least 15 minutes. Tubes were then centrifuged at 3,030 x g for 10 minutes at 4°C to pellet the DNA. The supernatant was removed and the pellet allowed to air dry for 10-25 minutes. The pellet was suspended in 2 ml sterile water. 1 ml of 7.5 M ammonium acetate was added, mixed and incubated on ice for 1 hour. Tubes were centrifuged at 12,000 x g for 30 min, the supernatants transferred to a fresh tube and 0.54 volumes of isopropanol were added, mixed and incubated at room temperature for at least 15 minutes. Tubes were then centrifuged at 5,930 x g for 10 min, the supernatant was removed and the pellet washed in 1 ml of 70% ethanol. Tubes were centrifuged at 5,930 x g for 10 min and all the ethanol was removed. The pellet was air dried for 20-30 minutes at room temperature and suspended in 0.5-1.0 ml of TE (10 mM Tris-HCl pH 7.5; 1mM EDTA) Finally, the DNA was treated with RNase A (5 µl of 1mg/ml stock).

2.2.3 PCR Reactions

Primers were designed to the upstream and downstream regions of the *A. fumigatus* AF293 2031 OR; cloning primer pair SEQ ID Nos. 46 (Ox9_for) and 47 (Ox10_rev). The following reagents and conditions were used:

PCR Master Mix

	10x high fidelity PCR buffer		5 µ1
	dNTP (clontech: 10mM)	v.	$1 \mu l$
	nH_2O		39 μ1
10	Pfu Ultra Polmerase (2.5U/μl)		1 μ1
	Forward primer (Ox9_for: 10 pmol/µl stock)		1 μ1
	Reverse primer (Ox10_rev: 10 pmol/µl stock)		1 μ1
	gDNA (1:30 dilution of stock)		2 ul

15 PCR Cycle

- 1) 95° C 2 min
- 2) 95° C 30 sec
- 3) 54° C 30 sec
- 4) 72° C 2 min
- 20 5) 72° C 10 min

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6) 8° C Hold

40 cycles of steps 2-4 were carried out and the PCR products were run on a gel. The product band (1.9kb) was excised from the gel and purified using Qiagen's QIAquick Gel Extraction Kit (Qiagen Ltd, Boundary Court, Gatwick Road, Crawley, West Sussex, RH10 9AX, UK) according to the manufacturers instructions and eluted into 30 μl of sterile water (BDH molecular biology grade/filter sterile).

2.2.4 Genomic DNA Cloning and Sequencing

Since the gDNA was amplified using Pfu ultra polymerase which produces blunt ends it was necessary to add 'A' overhangs before ligating in to pGEM Teasy. 12.5 μl of purified PCR product was incubated with 12.5 μl 2x PCR Reddy Mix (ABGene) at 70°

C for 30 minutes. The sample was then purified using Qigen Qiaquick gel extraction kit and eluted in 30 µl of molecular biology grade water.

The PCR product was then ligated into pGEM-Teasy (Promega) using the following ligation mixture:

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2x Buffer	5 μ1
pGEM Teasy	1 μ1
PCR product	3 μ1
T4 DNA Ligase	1 μl

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The reaction was incubated over-night at 4° C.

2 μ l of the ligation mix were then added to Select 96 cells (Promega) and incubated for 20 min on ice. Cells were then heat shocked at 42° C for 45 secs and placed back on ice. 250 μ l of room temp. SOC medium was then added and the cells incubated for 1 hour at 37° C, with shaking at 220 rpm. 50 and 200 μ l amounts were then plated on to LB agar plates containing ampicillin (100 μ g/ml), 50 μ l X-gal (4%) and 10 μ l IPTG (100 mM) and incubated over night at 37° C.

Individual white colonies were picked from each transformation inoculated into LB with ampicillin (100 μg/ml) and incubated over-night at 37° C, with shaking at 220 rpm. Plasmid DNA was extracted using Qiagen miniprep kit according to the manufacturers instructions. 1 μl of plasmid DNA was digested with EcoRI for 1 hour at 37° C. Fragment sizes were calculated to be 3Kb and 1.6Kb for gDNA and 3Kb and 1.2 Kb for cDNA. Clones showing the correct restriction digest pattern were sequenced at MWG Biotech UK Ltd, Waterside House, Peartree Bridge, Milton Keynes, MK6 3BY. The experimentally determined sequence of 2031 OR was identical in the coding regions to that identified by bioinformatic analyses (Example 2).

Example 3. cDNA sequencing and RACE for 2031 OR

The internal sequence of the 2031 OR message was experimentally determined by cloning and sequencing cDNA, and the 5' and 3' ends of the gene were determined by RACE (Rapid Amplification of cDNA Ends).

3.1 cDNA cloning and sequencing

3.1.1 Preparation of A. fumigatus RNA and cDNA

Fungal cultures were prepared as described in Example 2.2.2. Cultures were harvested by filtration, then washed twice with DEPC-treated water and transferred to a 50ml Falcon tube. Samples were frozen in liquid nitrogen and stored at -80°C until required.

To prepare RNA, fungal samples were ground to a fine powder under liquid nitrogen. RNA was then extracted using the Qiagen RNeasy Plant Mini Kit following the protocol for isolation of total RNA from filamentous fungi in the RNeasy Mini Handbook $(06/2001, \cdot)$ Pages 75-78, http://www.qiagen.com/literature/ handbooks/ma/mamini/1016272HBRNY 062001WW.pdf). The following modifications were used: At step 3, RLC was used as the lysis buffer of choice; At step 7, the Rneasy column was incubated for 5 min at room temperature after addition of RW1; The optional step 9a was carried out; At step 10, 30µl RNase-free water was added, the samples incubated for 10 min at room temperature, and then centrifuged; At step 11, the elution step was repeated to give a total volume of 60 µl RNA.

DNA contamination was removed from the RNA by the addition of Dnase, using 2 μl DNase per μg RNA, in the presence of 10X DNase buffer and incubating at 37°C for 2h. DNase-treated RNA was cleaned up using the RNeasy Plant Mini Kit following the RNeasy Mini Protocol for RNA Cleanup (RNeasy Mini Handbook 06/2001, pages 79-81).

To synthesise cDNA from the above RNA the following reaction mixture was prepared: 100ng-1μg of DNA-free RNA, 3μl oligo (dT) (100 ng/μl), and DEPC-treated water to a total volume of 42 μl. Samples were incubated in a heat block at 65°C for 5 min after which they were allowed to cool slowly to room temperature. Then 2μl Ultrapure dNTPs, 1μl reverse transcriptase (Stratascript) and 5μl 10X reverse transcriptase reaction buffer (Stratascript) were added. Samples were incubated at 42°C for 1h, denatured at 90°C for 5 min and then cooled on ice.

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PCR was carried out using the cDNA above to generate cDNA fragments using the primer pair SEQ ID No. 48 (Ox1_for) and SEQ ID No. 49 (Ox3_rev). PCR reactions were carried out using the following reagents and conditions:

5 PCR Master Mix

	10x high fidelity PCR buffer		5 μ1
	dNTP (clontech: 10mM)	•	1 µl
	MgSO ₄ (50 mM)		2 μΙ
	nH ₂ O		37.8µ1
10	Platinum TAQ Polmerase (5U/μl)		$0.2\mu l$
	Forward primer (Ox1_for: 10 pmol/µl stock)		$1\mu l$
	Reverse primer (Ox3_rev: 10 pmol/µl stock)		$-1 \mu l$
	cDNA		2 μ1

15 PCR Cycle

- 1) 94° C 5 min
- 2) 94° C 30 sec
- 3) 53° C 30 sec
- 4) 68° C 90 sec
- 20 5) 68° C 10 min
 - 6) 8° C Pause

Cycles 2-4 were run 40 times in total. The amplicon was 1269 bp. The PCR products were purified using Qiagen's QIAquick PCR Purification Kit (Qiagen Ltd, Boundary Court, Gatwick Road, Crawley, West Sussex, RH10 9AX, UK) according to the manufacturers instructions. The purified PCR products were examined on agarose gels.

PCR products were ligated into pGEM-Teasy, used to transform Select 96 cells, and sequenced as described in 2.2.4 above. The cDNA sequence obtained is given as bases 115 – 1385 of SEQ ID No. 2.

To determine the 5' and 3' ends of the genes, RACE (Rapid Amplification of cDNA Ends) was carried out, using the GeneRacerTM Kit (Invitrogen; cat. No. L1502-01), essentially as per manufacturers instructions.

5 3.2.1 Preparation of RNA

A. fumigatus biomass was prepared as described in 2.2.2. RNA was prepared using the FastRNA kit (QBIOgene) following the manufacturer's instructions (Revision 6030-999-1J05) with the following amendments: At step 1 40 mg of biomass was used per extraction; At step 2, samples were processed for 20 seconds at speed 5, incubated on ice for 3 minutes, and processed again for 20 seconds at speed 5; At step 3 samples were centrifuged for 5 minutes; At step 5, 500 μl DIPS were added, mixed, and incubated at room temperature for 2 minutes. Samples were mixed again and incubated for a further 2 minutes; At step 6 two washes in 250 μl SEWS were carried out; At step 7, the pellet was disolved in 50 μl SAFE buffer.

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3.2.2 RACE

1 μg total RNA prepared as described above was de-phosphorylated in a 10 μl reaction using 10 units of calf intestinal phosphate (CIP), 1 μl 10X CIP buffer and 40U RNaseOutTM (made up to 10 μl in DEPC water) at 50°C for 1 hour. Samples were then made up to 100 μl with DEPC water and the RNA extracted with 100 μl (25:24:1) phenol:chloroform: isoamyl alcohol. RNA was then precipitated by the addition of 2 μl mussel glycogen (10mg/ml), 10 μl 3M sodium acetate, pH 5.2 and 220 μl 95% ethanol and the sample frozen on dry ice for 10 minutes. RNA was pelleted by centrifugation at 14,500 rpm for 20 minutes at 4°C, washed with 70% ethanol, air dried and resuspended in 8 μl DEPC water.

De-phosphorylated RNA (7 μ l) was de-capped in a 10 μ l reaction with 0.5 U tobacco acid pyrophosphatase (TAP), 1 μ l 10x TAP buffer and 40U RnaseOutTM for 1 hour at 37°C. RNA was extracted with phenol:chloroform and precipitated as above, and then re-suspended in 7 μ l DEPC-treated water.

De-phosphorylated, de-capped RNA (7 μl) was added to the pre-aliquoted GeneRacerTM RNA Oligo (0.25 μg) and incubated at 65°C for 5 minutes. A 10 μl ligation reaction was then set up by the addition of 1 μl 10x ligase buffer, 1 μl 10mM

ATP, 40U RnaseOutTM and 5U T4 RNA ligase and incubated at 37°C for 1 hour. RNA was extracted and precipitated as described previously and re-suspended in 11 μl DEPC-treated water.

First-strand cDNA was prepared by the addition of 1 μl GeneRacerTM Oligo dT primer and 1 μl dNTP mix (10mM each) to 10 μl ligated RNA and incubated at 65°C for 5 minutes. The following reagents were added to the 12 μl ligated RNA and primer mix; 4 μl 5x first strand buffer, 2 μl 0.1M DTT, 1 μl RNaseOutTM and 1 μl SuperScriptTM II RT (200U/μl) and incubated first at 42°C for 50 minutes and then, to stop the reaction, at 70°C for 15 minutes. 2U RNase H was added to the reaction mix and incubated at 37°C for 20 minutes.

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To amplify the 5'cDNA ends a 50 μl PCR reaction was set up using 1 μl of the RACE-ready cDNA prepared above, 1 μl GeneRacerTM 5' primer, 1 μl reverse genespecific primer (SEQ ID No. 50; Ox6race_rev: 5 pmol/μl stock), 1 μl dNTP solution (10mM each), 2 μl 50 mM MgSO₄, 5 μl High Fidelity PCR buffer, 0.5 μl Platinum® Taq DNA Polymerase High Fidelity (5 U/μl) and 38.5 μl sterile water. Cycling parameters are given in Table II below.

A second, nested PCR stage was then set up using 1 μl of the RACE cDNA from the first stage above, 1 μl Nested 5' primer (supplied with kit), 1 μl reverse genespecific primer (SEQ ID No. 50; Ox6race_rev: 5 pmol/μl stock), 1 μl dNTP solution (10 mM each), 2 μl 50 mM MgSO₄, 5 μl High Fidelity PCR buffer, 0.5 μl Platinum® Taq DNA Polymerase High Fidelity (5 U/μl) and 38.5 μl sterile water. Cycling parameters are given in Table II below.

To amplify 3' ends a 50 μl PCR reaction was set up using 1 μl of the RACE-ready cDNA prepared above, 1 μl GeneRacerTM 3' primer (10 μM), 1 μl forward gene-specific primer (SEQ ID No. 51; Ox7race_for: 5 pmol/μl stock), 1 μl dNTP solution (10 mM each), 2 μl 50 mM MgSO₄, 5 μl High Fidelity PCR buffer, 0.5 μl Platinum® Taq DNA Polymerase High Fidelity (5 U/μl) and 38.5 μl sterile water. Cycling parameters are given in Table II below:

A second, nested PCR stage was then set up using 1 µl of the 3' RACE cDNA from the first stage above, 1 µl Nested 3' primer (supplied with kit), 1 µl reverse genespecific primer (SEQ ID No. 52; Ox8race_for: 5 pmol/µl stock), 1 µl dNTP solution (10mM each), 2 µl 50 mM MgSO₄, 5 µl High Fidelity PCR buffer, 0.5 µl Platinum®

Taq DNA Polymerase High Fidelity (5U/ μ l) and 38.5 μ l sterile water. Cycling parameters are given in Table II below.

Table II. Cycling parameters for 5' and 3'RACE

	· · · · · · · · · · · · · · · · · · ·					200
5' and 3'	RACE	· · ·)	Nested 1	PCR	300	
94 °C	2min	1 cycle	94° C	2 min	1 cycle	
. *				X	- (
94 °C	30s	5 cycles	94° C	30 sec	25 cycles	
72 °C	1min	•	67° C	30 sec		
			68° C	1 min		
94°C	30s	5 cycles				
70 °C	1min					
	• - ()		68° C	10 min	1 cycle	
94°C	30s	25 cycles	8° C	Hold	•	
64 °C	30s	•				
68 °C	1min				٠	
68 °C	10min	1 cycle				
8°C	Hold ·					
			L		1	

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5' and 3' RACE confirmed the predicted 5' ATG and 3' stop codon as well as giving the 5' and 3' untranslated regions shown as bases 1-114 and 1385 – 1921 of SEQ ID No. 2. The coding sequence for 2031 OR thus determined was identical to that given as bases 299-469 and 520-1618 of the gDNA gien as SEQ ID No. 1.

Example 4. Identification of other fungal 2031 ORs and related genes

Homologs of A. fumigatus 2031 OR were identified in other fungi and bacteria by means of bioinformatics analysis. Sequences identified by bioinformatics can be used to design primers which in turn can be used in PCR to generate DNA coding for the 2031 OR homolog.

Alternatively, degenerate PCR can be used to obtain sequence for novel genes, which can then be used to generate probes for screening cDNA or genomic libraries of the organism of interest to identify clones containing the 2031 OR homolog. As a further alternative, Southern blots using fragments of genes from one species as probes can be used to identify the presence of a homolog in the genome of a second species. The same probe can then be used to screen cDNA or genomic DNA libraries. Once clones corresponding to the novel genes have been identified they can be expressed for functional characterisation of the protein.

4.1 Identification of homologs by bioinformatics

Analysis of the 2031 OR protein sequence with PFAM (http://www.sanger.ac.uk/Software/Pfam/) identified this as a member of the Oxidored FMN family (PF00724), E-value 3.6e-57. This includes the well-characterised "Old Yellow Enzyme" proteins of *S. cerevisiae* and other fungi.

Homologs of A. fumigatus 2031 OR sequence were identified by database searches (see Table III). Where necessary, matching contigs were down-loaded and genes predicted from genomic DNA by Genscan analysis, blast searches, alignment and visualisation with Artemis as described in Example 2. Protein and nucleotide multiple alignments were generated for 2031 OR and related genes (Figures 1 and 2).

Protein and nucleic acid multiple alignments are generated by means of programs such as ClustalX (Thompson et al., 1994, Nucleic Acids Research, 22, 4673-4680; Thompson et al., 1997, Nucleic Acids Research, 24, 4876-4882;) and/or using manual alignment editors such as Align (Hepperle, D., 2001: Multicolor Sequence Alignment Editor. Institute of Freshwater Ecology and Inland Fisheries, 16775 Stechlin, Germany).

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Contig/EST/	E-	SEQ ID No.	, · ·		Species (details of search
predicted	value ¹	EST/gDNA	CDNA ²	Protein	given in footnotes)
gene		Y			
4929	6.6e-81	4	5	6	Aspergillus fumigatus ³
4951	1.1e-68	7 .	-	. 8	Aspergillus fumigatus³
4875	5.7e-13	-	-	-	Aspergillus fumigatus ³
4961	3.2e-10	- 0	_	-	Aspergillus fumigatus ³
1.112	3e-33	9	-	10	Aspergillus nidulans ⁴
6-2431	2.6e-77	11	·-	12	Candida albicans ⁵
6-2464	5.9e-50	13	-	14	Candida albicans ⁵
6-2460	5.8e-19	-	-	-	Candida albicans ⁵
A36990	1e-15	-	_		Candida albicans ⁶
NCU07452.1	7e-94	15	-	16	Neurospora crassa ⁷
NCU08900.1	2e-19	17	18	19	Neurospora crassa ⁷
NCU04452.1	2e-23	-	-	_	Neurospora crassa ⁷
MG04569.3	1e-106	20	21	22	Magnaporthe grisea ⁸
MG03823.3	8e-19	43	-	44	Magnaporthe grisea ⁸
NP_595868	1e-05	23		24	Schizosaccharomyces
			į		pombe ⁶
OYE1	1e-15	-	-	-	Saccharomyces cerevisiae ⁶
OYE2	4.5e-19	-	-	-	Saccharomyces cerevisiae ⁹
OYE3	1.0e-16	-	-	-	Saccharomyces cerevisiae9
FsCon[0063]	1e-82	28	29	30	Fusarium
(EST contig)					sporotrichioides ¹⁰
Gz15771741	5e-76	36	37	38	Fusarium graminearum ¹⁰
					0 .
Mg[0281]	2e-67	39		40	Mycosphaerella
(EST contig)					graminicola ¹⁰
CtCon[0249]	1e-55	25	26	27	Colletotrichium trifolii ¹⁰
(EST contig)	·				
FsCon[0458]	1e-42	34		35	Fusarium

					70
(EST contig)					sporotrichioides ¹⁰
FsCon[0237]	1e-40	31	32	33	Fusarium
(EST contig)					sporotrichioides ¹⁰
Mga0328f	3e-35	41	-,0	42	Mycosphaerella
		,			graminicola ¹⁰
T44612	1e-52	-	-	_	Pseudomonas putida ¹¹
NP_625402	1e-79	-	_	-	Streptomyces coelicolor ¹¹
NP_295913	1e-78	-		-	Deinococcus radiodurans ¹¹
AF320254	5e-55	-	j - 0	-	Deinococcus radiodurans ¹¹
FG00074.1		82	82	83	Fusarium graminearum ¹²
Contig 1.2	1e-71	84	84	<i>85</i>	Ustilago maydis ¹³

¹E-values for blast scores refer to searches with 2031 OR protein unlesss pecified otherwise in footnotes.

²A cDNA was generated in cases where either the gene contains multiple exons, or there are probable frame-shift errors from sequencing of the EST, or the EST given is the non-coding strand.

³Search of the *A. fumigatus* genome at http://www.TIGR.org (tblastn) with NP 595868.

⁴Search of A. nidulans genome held on local machine (tblastn).

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⁵Search of the *C. albicans* genome at http://www-sequence.stanford.edu/group/candida/ (blastp).

⁶Search of the non-redundant protein sequence database (nr) at http://blast.genome.ad.jp (blastp).

⁷Search of the N. crassa predicted proteins at

http://www.broad.mit.edu/annotation/fungi/neurospora/ (blastp).

⁸Search of the *M. grisea* predicted proteins at http://www.broad.mit.edu/annotation/fungi/magnaporthe/ (blastp).

⁹Search of *S. cerevisiae* orf proteins (http://mips.gsf.de/cgi-bin/blast/blast page?genus=yeast)

20 ¹⁰Search of COGEME pathogenic fungal EST database at http://cogeme.ex.ac.uk/blast.html (tblastn, max E-val=0.1).

¹¹Search of NCBI non-redundant protein database on local machine with SEQ ID No.

1 (blastx). Only a selected set of hits against bacterial proteins are shown.

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To clarify the relationships between the 2031 OR, OYE and the hits identified from blast searches, phylogenetic analysis was carried out. The PHYLIP suite of programs was used (Felsenstein, Felsenstein, J., 2002. PHYLIP (Phylogeny Inference Package) version 3.6a3. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle). The multiple alignment used for the analyses was essentially that given in Figure 1 with partial sequences, gapped regions and unreliably aligned sections excluded. A distance matrix was generated using PROTDIST with the Jones-Taylor-Thornton model and the tree inferred using FITCH with global rearrangements and 10 jumbles of input order. 100 bootstrap replicates were generated using SEQBOOT, distance matrices generated using PROTDIST as above, trees inferred using NEIGHBOUR, and then bootstrap values and the consensus tree were calculated using CONSENSE. Trees were viewed using TREEVIEW (Page, 1996 Page, R. D. M., 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12, 357-358.)

Phylogenetic analysis identified a clade supported by good bootstrap values, which included A. fumigatus 2031 OR and other enzymes. This could be distinguished from a clade containing OYE enzymes which was also supported by good bootstrap values. Bacterial homologs of both 2031 OR and OYE (not shown) were also identified. We have therefore identified a set of 2031 OR homologs which, surprisingly, is distinct from the well-characterised OYE family, and which, by virtue of the essentiality demonstrated for A. fumigatus 2031 OR, represents a set of potential targets for anti-fungal drugs

30 <u>4.2 Identification of homologs by degenerate PCR</u>

4.2.1. Preparation of genomic DNA from organism of interest

¹²Search of F. graminearum predicted proteins held on local machine (blastp).

¹³Search of *U. maydis* contigs held on local machine (tblastn).

Fungal cultures are prepared using methods suitable for particular species. For example, Aspergillus and Candida species, *Cryptococcus neoformans, Fusarium solani* and *Trichophyton* species are maintained on Sabouraud dextrose agar at 30-35°C; *Leptosphaeria nodorum* on Malt agar medium (30 g/L malt extract; 15 g/L Bacto-agar, pH 5.5), 24.0°C; *Magnaporthe grisea* on Oatmeal agar (6.7 g/L agar, 53.3 g/L instant oatmeal) 25.0°C, or Cornmeal agar (Difco 0386), 26.0 C; *Phytophthora capsici* cultures were maintained on on V-8 agar at 24°C; *Pyricularia oryzae* cultures were maintained on rice polish agar at 24°C under white fluorescent lights (12hr artificial day), and were subcultured every 7 - 14 days by the transfer of mycelial plugs to fresh plates; *Pythium ultimum* cultures were maintained on PDA at 24°C, and subcultured every 7 days by the transfer of aerial mycelium to fresh plates with an inoculating needle; *Rhizoctonia solani* cultures were maintained on PDA at 24°C under fluorescent lights (12 h artificial day), and subcultured every 7 days by the transfer of mycelial plugs to fresh plates; *Ustilago maydis* cultures were maintained on PDY agar at 30°C in the dark, and subcultured by re-streaking.

Genomic DNA was prepared from cultures using standard methodologies, e.g. using the Qiagen DNeasy Plant Kit, or using methods described in Example 2.2.

4.2.2 PCR

- Primers (SEQ ID Nos. 53 and 54) were designed on the 2031 OR-specific regions given as regions 2 and 6 in Figure 2. However, those skilled in the art will appreciate that it may be necessary to try alternative primers. PCR reactions using the above primer pair are set up as follows:
- 12.5 μl 2x ReddyMix PCR mastermix (ABIgene)
 1 μl primer SEQ ID No. 53 (5 pmol)
 1 μl primer SEQ ID No. 54 (5 pmol)
 template gDNA (1.5-4 μg/ml)
 nuclease-free water to give a final volume of 25 μl

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The reactions are run using the following conditions on a Biometra personal PCR cycler (Thistle Scientific Ltd, DFDS House, Goldie Road, Uddington, Glasgow, G71 6NZ):-

.5	Step1	95°C	5min
	Step2	95°C	1min
,	Step3	53°C	1min 30sec
	Step4	68°C	2min 30sec
	Step5	72°C	10min
10	Step6	4°C .	Hold

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30 cycles of steps 2-4 are carried out. The PCR products are purified (to remove residual enzymes and nucleotides) using Qiagen's QIAquick PCR Purification Kit (Qiagen Ltd, Boundary Court, Gatwick Road, Crawley, West Sussex, RH10 9AX, UK) according to the manufacturers instructions and eluted into 40µl of sterile water (BDH molecular biology grade/filter sterile). The purified PCR products are examined on 1% agarose gels.

Those skilled in the art will appreciate that degenerate PCR may require variations in a number of parameters in the attempts to generate a product. These include primer concentration, template concentration, concentration of Mg²⁺ ions, elongation and annealing times, and annealing temperature. Variations in temperature can be accommodated by the use of a gradient PCR machine.

The purified PCR products are cloned into pPEM-Teasy (Promega) and then transformed into XL10-Gold® Kan ultracompetent *E. coli* cells according to the manufacturer's instructions. The transformation reactions are then plated onto LB agar plates containing ampicillin (100 μg/ml), 50 μl X-gal (4%) and 10 μl IPTG (100 mM). Following overnight incubation at 37°C, individual white colonies from each transformation are sub-cultured into LB broth containing ampicillin (100 μg/ml). After overnight incubation at 37°C with shaking, plasmids are extracted using Qiagen spin mini plasmid extraction kits according to the manufacturers instructions and sent away for full-length sequencing.

4.3 Identification of homologs by Southern Blotting

4.3.1 Digestion of genomic DNA and transfer to nylon membranes

Genomic DNA from the fungi of interest are digested with the appropriate restriction enzyme and run on 0.8 % agarose gel. The gel is then submerged in 250 mM HCl for no more than 10 mins, with shaking, at room temperature, after which the gel is rinsed with sterilised RO water.

Transfer of the DNA onto nylon membrane is carried out using 0.4 M NaOH. Transfer protocols and apparatus are well known and are described in e.g. Sambrook et al., (1989), Molecular Cloning, 2nd Edition., Cold Spring Harbor Laboratory Press. After transfer, the DNA is fixed to the membrane by baking at 120°C for 30 min. The membrane can then be used immediately, or stored dry for future use.

15 4.3.2. Preparation of probe

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Probes are generated either by restriction digests of DNA or by PCR of an appropriate region. A suitable probe can be generated by PCR using the primer pair SEQ ID Nos. 53 and 54, A. fumigatus genomic DNA, and the methods give in 4.2.2.

1 μg DNA template is diluted in molecular biology water to a total volume of 16 μl, denatured in a boiling water bath for 10 mins, and quickly chilled on ice. 4 μl DIG-High Prime (1 mM dATP, 1mM dCTP, 1mM dGTP, 0.65 mM dTTP, 0.35 mM alkalilabile-digoxygenin-11-dUTP, 1 U/μl labelling grade Klenow enzyme, 5 x reaction buffer, in 50% (v/v) glycerol) is then added and the reaction incubated at 37°C for 20 hours, after which 2 μl of 200 mM EDTA pH 8.0 is added to terminate the labelling reaction. The labelling efficiency is estimated by comparison with DIG-labelled control DNA.

4.3.3. Prehybridisation and Hybridisation

The membrane is placed in a hybridisation tube containing 20 ml of prehybridisation solution (DIG Easy Hyb, Roche) per 100cm² of membrane surface area and prehybridised at 42°C for 2 hours in a hybridisation oven. The DIG- labelled probe is denatured by heating in a boiling water bath for 10 min and then chilled directly on

ice. The probe is then diluted to ~200 ng/mL in hybridisation solution (Easy Hyb, Roche; at least 5 mL of hybridisation solution is required per hybridisation). The prehybridisation solution is discarded from the hybridization tube and the hybridisation solution containing the DIG-labelled probe added quickly. The hybridisation then proceeds overnight at a 42°C in the hybridisation oven. The optimum temperature is dependant on probe size and homology with target sequence and was determined empirically.

After hybridisation, the membrane is washed twice at 42°C, 5 mins per wash, with 50 mL of stringency wash solution (3 x SSC, 0.1% SDS; where 20 x SSC buffer is 3 M NaCL, 300mM sodium citrate, pH 7.0), followed by two washes at RT, 15 min per wash, in 50 mL stringency wash solution. The stringency of these washes can be decreased by increasing the SSC concentration to 6 x SSC, 0.1% SDS and/or decreasing the wash temperatures.

15 4.3.4. Detection

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The membrane is washed in 20 mL washing buffer (100mM Maleic acid, 150 mM NaCl; pH 7.5; 0.3% v/v Tween 20), and then incubated successively with the following; 20 mL blocking solution (1 % w/v blocking reagent for nucleic acid hybridisation, Roche, dissolved in 100mM maleic acid, 150 mM NaCl, pH 7), for 30 min at room temperature; Anti-DIG-alkaline phosphatase (Roche) diluted 1:5,000 in blocking buffer, 30 min at room temperature; Washing buffer, two washes each of 15 min at room temperature; Detection buffer (100 mM Tris-Hcl, 100 mM NaCl; pH 9.5), 2 min at room temperature. The membrane is then removed, placed on top of an acetate sheet, and ~ 0.5 ml (per 100cm²) of CSPD or CDP-star added to the top of the membrane. A second sheet of acetate is then placed over the surface of the membrane, the assembly incubated for 5 min at room temperature and then sealed in a plastic bag. The assembly is then exposed to X-ray film for between 15 min and 1 hour. Optimal exposure time is determined empirically by increasing exposure time up to 24 hours.

The presence of a band on the gel is evidence of a gene in the genomic DNA of interest. The molecular weight of the band depends on the size of the restriction fragment that contains the gene.

Example 5. Expression during infection of wax moth larvae (Galleria melonella) and mice with A. fumigatus

5 5.1 Preparation of cDNA from infected wax-moth larvae

Wax moth larvae have been shown to be good model systems in which to study *Candida* infection (Cotter et al., 2000, FEMS Immunol Med Microbiol 27, 163-9; Brennan et al., 2002, FEMS Immunol Med Microbiol 34, 153-7). We have found that this insect system is also a good system in which to study Aspergillus infection (D.

Law and J. Rooke, manuscript in preparation).

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5.1.1 Growth and infection of wax-moth larvae

Spores of A. fumigatus (AF293), grown on Sabaraud Dextrose agar, were harvested and re-suspended in PBS/Tween 80. Spores were washed and the concentration adjusted such that a 10 µl inoculum will cause death in 90% of the test group 3-4 days after infection (for AF293 this is 5.0-7.0x10⁸ cfu/ml). Inoculum concentration was estimated using an improved Neubauer haemocytometer counting chamber and confirmed by TVC enumeration.

Wax moth larvae were purchased from Livefood UK, Somerset, UK (www.livefood.co.uk), and were maintained in the dark at room temperature in wood shavings prior to infection. Healthy larvae (250 mg +/- 50 mg) were selected and incubated at 4°C for 10 minutes immediately prior to infection to immobilise them. Larvae were then injected through the cuticle of the left last pro-leg with 10 μl spore suspension (100x stock), using a sterile Hamilton syringe. Larvae were then transferred to a sterile Petri dish. The following controls were also established: Larvae injected with 10 μl PBS/Tween only; larvae injected with 10 μl heat killed spores (killed by incubation for 20 min 100°C); larvae pierced but not injected; and untouched larvae. Larvae were incubated at 30°C and monitored at least twice daily. All treatments and controls were carried out on batches of 10 larvae. Larval deaths and general health condition was recorded every 24 hrs and dead or moribund larvae were removed from the test group.

5.1.2 Preparation of DNA-free RNA from Aspergillus fumigatus-infected wax moth larvae (Galleria melonella).

cDNA was prepared from the following sources: Uninfected larvae; larvae after 48h infection with A. fumigatus (early infection); larvae after 72h infection with A. fumigatus (late infection); larvae infected with heat-killed A. fumigatus spores; and A. fumigatus grown in Sabaraud Dextrose agar broth for 16hr.

Frozen larvae were ground to a fine powder under liquid nitrogen in a mortar and pestle previously baked at 22°C overnight, treated with RNaseZAP, rinsed with DEPC-treated water (0.1% (v/v) DEPC, stirred for 1h and autoclaved for 1h) and cooled with liquid nitrogen. Ground sample was transferred to Eppendorf tubes (no more than 50 mg per tube) and total RNA extracted using the Qiagen RNeasy Plant Mini Kit following the protocol for isolation of total RNA from filamentous fungi in the RNeasy Mini Handbook (06/2001, Pages 75-78, http://www.qiagen.com/literature/handbooks/

15 ma/mamini/1016272HBRNY_062001WW.pdf).

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The following modifications were used: At step 3, 600 µl RLT was added to each 50 mg tissue and vortexed; At step 4, samples were centrifuged for 3 min at maximum speed; At step 6, all samples from the same tissues were applied to the same RNeasy column; At step 7, RNeasy column was incubated for 5 min at room temperature after addition of RW1; Optional step 9a was carried out twice; At step 10, 30 µl RNase-free water was added, samples incubated for 10 min at room temperature, and then centrifuged for 1 min at 14,000 RPM; At step 11, the elution step was repeated to give a total volume of 60 µl RNA. A sample of the RNA was run on a 1.5% agarose gel and the amount of RNA quantified using the molecular marker. RNA was then stored at -80°C.

A portion of the RNA was Dnase treated using 2 µl RNase-free DNase (Promega) per µg RNA, in the presence of 10X DNase buffer (Promega) at 37°C for 4h. The RNA was then cleaned up using the Qiagen RNeasy Plant Mini Kit following the RNeasy Mini Protocol for RNA Cleanup (RNeasy Mini Handbook 06/2001, pages 79-81), but including a further DNase treatment step during clean-up as in the Rneasy handbook.

The following modifications were made: Optional step 5a was carried out; At step 6, 30µl RNase-free water was added, samples incubated for 10 min at room temperature and then centrifuged for 1 min at 14,000 RPM; At step 7, the eluate from step 6 was transferred onto the RNeasy column, incubated for 10 min at room temperature, and then centrifuged for 1 min at 14,000 RPM. A sample of the DNase-treated RNA was run on an agarose gel, quantified and stored at -80°C.

5.1.3 Checking RNA samples for DNA contamination

To verify the absence of genomic DNA from the RNA samples, PCR was carried out using primers that amplify the β -tubulin gene (SEQ ID Nos. 77 and 78). In the absence of a reverse-transcription step, only gDNA will be detected and thus any gDNA contamination will be revealed. The following reaction mixture was set up:

12.5 µl 2x ReddyMix PCR mastermix (ABIgene)

1 μl each primer (5 pmol)

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15 template gDNA (1.5-4 μg/ml)

nuclease-free water to give a final volume of 25 µl

The reactions were run using the following conditions on a Biometra personal PCR cycler (Thistle Scientific Ltd, DFDS House, Goldie Road, Uddington, Glasgow, G71 6NZ):-

	Step1	95°C	5min
	Step2	90°C	1min
	Step3	51°C	1min
25	Step4	68°C	1min
	Step5	68°C	10min
	Step6	4°C	Hold

40 cycles steps 2-4

If a PCR product was observed, genomic DNA was present and the sample was 30 DNase-treated again. If the PCR was negative, no DNA was present in the sample.

5.1.4 Preparation of cDNA

300 μg DNA-free RNA and 3 μl oligo (dT) (100 ng/μl) were added to an RNase-free 0.5 ml microcentrifuge tube, and made up a total volume of 42 μl with DEPC-treated water. Samples were mixed and incubated in a heat block at 65°C for 5 min and then slowly cooled to room temperature. 2 μl Ultrapure dNTPs (10 mM each, Clontech), 1 μl stratascript reverse transcriptase (Stratagene) and 5 μl 10X reverse transcriptase reaction buffer were then added. The samples were incubated at 42°C for 1h, denatured at 90°C for 5 min and then cooled on ice. Samples were dispensed in 5-10 μl aliquots and stored at -20°C.

10 <u>5.2. Preparation of cDNA from infected mice</u>

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5.1.1 Infection of mice with A. fumigatus and extraction of tissues.

Mice were infected with Aspergillus fumigatus and organs harvested as follows. Thirteen male CD1 mice were injected with the immunosuppressant cyclophosphamide (0.025 g/ml; 200 mg/kg) IV via the tail vein. After 72 hours, twelve mice were injected with 0.15 ml Aspergillus fumigatus AF293 conidia (7.5 x 10⁵/ml). 11 hours after infection, four mice were sacrificed with an overdose of inhaled halothane. The brain, lungs, liver and kidney were removed, frozen by immersion in liquid nitrogen, and stored at -70°C. A further four mice were also sacrificed at 24 and 48 hours after infection.

RNA was prepared from mouse tissues as described for wax moth larvae above (5.1.2 and 5.1.3).

5.2.2 Preparation of cDNA from DNA-free RNA.

cDNA was prepared from DNA-free RNA using the Promega Reverse Transcription kit, following the protocol as supplied with the product (Technical Bulletin No. 099, http://www.promega.com/tbs/tb099/tb099.pdf). In a modification to the protocol, the cDNA synthesis reaction was incubated for 60 min at 42°C rather than for the suggested 15 min. Samples were stored in 5-10µl aliquots at -20°C.

30 <u>5.3 Design and optimisation of primers</u>

Primers were designed against the 2031 OR cDNA sequence using Beacon Designer 2.1 (Premier Biosoft, http://www.premierbiosoft.com) with the following parameters;

Target $Tm = 58 \pm 8$ °C; Length of primers = 16-24; Amplicon length = 75-150 bp. All other settings were default. Care was taken to choose primers that would not form dimers or other secondary structures. Secondary structures of amplicons were calculated using mfold

(http://www.bioinfo.rpi.edu/applications/mfold/old/dna/form1.cgi) and primer sets giving an amplicon with little or no secondary structure were chosen. The resulting primers are given as SEQ ID Nos. 79 and 80.

To determine optimum annealing temp for the primer set, a gradient PCR was run on an Icycler PCR machine (Biorad), using A. fumigatus AF293 genomic DNA as a template and the following reaction mixture:

112.5 μ l Abgene PCR Reddymix 9 μ l SEQ ID No. 79; OXRED 2031F6 (5 pm/ μ l) 9 μ l SEQ ID No. 80; OXRED 2031R5 (5 pm/ μ l) 85.5 μ l H₂O

For the negative control, the gDNA was omitted and the amount of water increased correspondingly.

For each mix, 25 µl was pipetted into 8 wells on a multiwell plate, and each well run at a different temp (between 50 and 65°C) with the following conditions:

Step 1. 95° C -5 min

9 μl AF293 gDNA (10 ng/ul)

25 Step2. 95°C - 1 min

Step3. Gradient 50-65°C - 1.5 min

Step4. 72°C - 1 min

Step5. 72°C - 10 min

Step6. 8°C - hold

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Steps 2-4 were run for 30 cycles

The PCR products were run on a 2% agarose gel. A single band of the correct size of 148 bp was seen on the gel for all the temperatures, and the optimum was found to be 63°C.

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5.4 Testing species-specificity of primers

The real-time primers designed above were further tested to ensure that mouse nucleic acid was not amplified using these primers. Four reactions were set up, each containing the following:

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12.5 µl Abgene Reddymix

1 μl primer SEQ ID No. 79

1 μl primer SEQ ID No. 80

9.5 µl H2O

and either; 1 μl infected mouse kidney cDNA (50 ngμl; experimental); 1 μl uninfected mouse kidney cDNA (50 ng/μl; uninfected control); 1 μl AF293 gDNA (10 ng/μl; positive control); 1 μl water (negative control).

The following PCR settings were used:

20 Step1 95°C - 5 min

Step2 95°C - 1 min

Step3 63°C – 1.5 min

Step4 $72^{\circ}C - 1 \min$

Step5 72° C - 10 min

25 Step6 8°C - hold

Steps 2-4 were run 40 times

The PCR products were run on a 2% agarose gel. A. fumigatus genomic DNA gave a band of 148 bp, the expected size, but no bands were seen in uninfected or infected mouse cDNA. These primers therefore appeared to be specific.

5.5 Real-time PCR to detect expression in infected larvae

PCR reactions were set up using the Biorad iQ SYBR green supermix as follows:

14 μl Primer SEQ ID No. 79
14 μl Primer SEQ ID No. 80
175 μl SYBR mix
133 μl H₂O

Step1.

10 Four reactions were set up containing 72 μl of the above mix and either; 3 μl H₂O; 3 μl uninfected larvae cDNA (50 ng/μl); 3 μl AF293 gDNA (5 ng/μl); or 3 μl infected larvae cDNA (50 ng/μl) were added. 3 x 25 μl aliquots of each reaction were aliquoted into an Abgene multiwell plate, the plate sealed with optical sealing tape (Biorad), then placed in a Biorad Icycler real-time PCR machine. Reactions were run with the following conditions:

3 min

	L		
	Step2.	95.0°C	30 sec
	Step3.	63.0°C	30 sec
20	Data collection and re	al-time analysis enabled.	
	Step4.	72.0°C	15 sec
	60 cycles of steps 2-4.	•	
	Step5.	95.0°C	30 sec
	Step6.	50.0°C	30 sec
25	Step7.	50.0°C	10 sec

95.0°C

90 cycles of step 7 with setpoint temperature increased by 0.5°C after each cycle starting with cycle 2. Melt curve data collection and analysis enabled.

Results are shown in Tables IV and V. Expression of 2031 OR was demonstrated in both Af293 cDNA (Ct = 25.8) and in infected larvae (Ct = 32.3). Therefore, the message is expressed both in A. fumigatus cultures and in A. fumigatus from infected

larvae. The negative and uninfected larvae controls give only primer dimers and non-specific products.

Table IV. PCR Quantification Spreadsheet Data for SYBR-490

Well	Identifier	Ct
C08	infected larvae (50ng)	33
C09	infected larvae (50ng)	32.4
C10	infected larvae (50ng)	31.4
D03	Negative	51.3
D04 .	Negative	N/A
D05	Negative	55.6
H03	uninfected larvae	36.4
H04	uninfected larvae	N/A
H05	uninfected larvae	N/A
H08	A. fumigatus gDNA (5ng)	25.8
H09	A. fumigatus gDNA (5ng)	26
H10	A. fumigatus gDNA (5ng)	25.8

Data Analysis Parameters: Calculated threshold was replaced by the user selected threshold 7.4.; User selected baseline cycles were 2 to 10.

Table V. Melt Curve Analysis Spreadsheet Data for SYBR-490

Well	Well Identifier	Peak ID	Melt Temp
C8	infected larvae (50ng)	C8.1	88.5
C9	infected larvae (50ng)	C9.1	88.5
C10	infected larvae (50ng)	C10.1	88.5
D3	Negative	D3.1	78 .
D5	Negative	D5.1	81.5
		D5.2	77.5

H3	uninfected larvae	H3.1	81.0
H5	uninfected lärvae	H5.1	78.0
H8	A. fumigatus gDNA (5ng)	H8.1	89.0
H9	A. fumigatus gDNA (5ng)	H9.1	89.0
H10	A. fumigatus gDNA (5ng)	H10.1	89.0

Melt Curve Analysis Parameters; Threshold for automatic peak detection was set at 2.64.

5.6 Real-time PCR to detect expression in infected mouse kidney cDNA.

Real-time experiments similar to those described in 5.5 using 1 μ l of infected mouse cDNA showed no amplification (data not shown). The experiment was therefore carried out using an increased amount of infected mouse cDNA with the following conditions:

18 μl Primer SEQ ID No. 79
18 μl Primer SEQ ID No. 80
15 225 μl SYBR mix
99 μl H₂O

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Four reactions were set up containing 60 μ l of the above mix and either; 15 μ l H₂O; 3 μ l uninfected mouse kidney (50 ng/ μ l) + 12 μ l H₂O; 15 μ l infected mouse kidney – 48h post-infection (50ng/ μ l); or 3 μ l AF293 cDNA (5ng/ μ l) + 12 μ l H₂O were added. 3 x 25 μ l aliquots of each reaction were aliquoted into an Abgene multiwell plate, the plate sealed with optical sealing tape (Biorad), then placed in a Biorad Icycler real-time PCR machine. Reactions were run with the following conditions:

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 Step1.
 95.0°C
 3 min

 Step2.
 95.0°C
 for 30 sec

 Step3.
 63.0°C
 for 30 sec

Data collection and real-time analysis enabled.

Step4.

72.0°C

for 15 sec

60 cycles of steps 2-4.

Step5.

.95.0°C

for 30 sec

Step6.

50.0°C

for 30 sec

Step7.

50.0°C

for 10 sec

90 cycles of step 7 with setpoint temperature increased by 0.5°C after each cycle starting with cycle 2. Melt curve data collection and analysis enabled.

Expression of A. fumigatus AF293 2031 OR was seen in cDNA (Ct = 28.8) but only in 2 of the 3 infected mouse kidney reactions (Ct values = 34.4, 41.2) (Tables VI and VII). The product in the other infected kidney cDNA reaction (well A12) was a primer dimer or a non-specific product (Tm = 81°C on the melt curve), whereas the correct 2031 OR product has a Tm of 88.5°C (Tables VI and VII). The negative and uninfected kidney controls gave only primer dimers and non-specific products.

Table VI: PCR Quantification Data for SYBR-490

Well	Identifier	Ct
A10	infected kidney (250ng)	34.4
A11	infected kidney (250ng)	
A12		41.2
	infected kidney (250ng)	38
D02	Negative	50.3
D03	Negative	54.6
D04	Negative	46.2
H02	uninfected kidney	52.8
H03	uninfected kidney	54
H04	uninfected kidney	51.8
H10	AF293 (5ng)	28.7
H11	AF293 (5ng)	28.7
H12	AF293 (5ng)	30

Calculated threshold was replaced by the user selected threshold 5.4. User selected baseline cycles were 2 to 10.

Table VII. Melt Curve Analysis Spreadsheet Data for SYBR-490

Well	Well Identifier	Peak ID	Melt Temp
A10	infected kidney (250 ng)	A10.1	88.5
A11	infected kidney (250 ng)	A11.1	88.5
A12	infected kidney (250 ng)	A12.1	81.0
.D2	Negative	D2.1	79.0
D3	Negative	D3.1	78.0
D4	Negative	D4.1	78.0
H2	uninfected kidney -	H2.1	78.5
H3	uninfected kidney	H3.1	77.5
H4	uninfected kidney	H4.1	90.5
H10	AF293 (5ng)	H10.1	88.5
H11	AF293 (5ng)	H11.1	88.5
H12	AF293 (5ng)	H12.1	88.5

Threshold for automatic peak detection was set at 2.09.

A. fumigatus 2031 OR is therefore clearly expressed during infection of wax moth larvae. 2031 OR is only expressed at a very low level during infection of mouse kidney, since increased amounts of template had to be used to give a signal. The expression during infection suggests that the gene product may be a suitable target for an anti-fungal drug.

Example 6. Expression of recombinant 2031 OR and/or fragments

Recombinant proteins or fragments were expressed to enable detailed study of function and as the starting point for the development of a high-throughput screen for inhibitory compounds.

6.1 Production of cDNA constructs

PCR was carried out using cDNA prepared as described above to generate polynucleotides encoding 2031 OR sequence essentially corresponding to SEQ ID No.

3. PCR reactions were carried out using the following reaction mixture and conditions.
All Reagents were present in the KOD kit (Novagen).

2.5 µl 10x PCR Buffer

5 μl dNTPs (2mM)

10 2 μl MgSO₄ (25mM)

1 μl primer A (5 pmol) (SEQ ID No. 55; SL_OxXa30F5)

1 µl primer B (5 pmol) (SEQ ID No. 56; SL-OxXa30R7)

1 μl template cDNA

11.5 µl nuclease-free water

15 1 μl KOD Polymerase

PCR reactions were run using the following conditions:-

	Step1	94°C	5 min
20	Step2	94°C	1 min
	Step3	59.3°C	1 min
	Step4	68°C	1 min 30sec
	Step5	68°C	10 min
	Step6	10°C	Hold

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40 cycles of steps 2-4 were carried out and the PCR products were purified using Qiagen's QIAquick PCR Purification Kit (Qiagen Ltd, Boundary Court, Gatwick Road, Crawley, West Sussex, RH10 9AX, UK) according to the manufacturers instructions. The purified PCR products were examined on agarose gels.

cDNA fragments were then cloned in to the pET30 Xa/LIC vector (Novagen), transformed into Nova Blue chemically competent *E. coli* cells, and plated on to a prewarmed kanamycin (+) selection plate. After an overnight incubation at 37° C,

kanamycin-resistant colonies were selected and grown up in kanamycin containing LB medium. Plasmid DNA was isolated using the Plasmid Mini Kit (Qiagen). Confirmation of the presence and correct orientation of the inserts was determined by restriction analysis and sequencing of the construct.

Purified plasmid DNA, which had been confirmed to be of the correct sequence and orientation, was transformed into chemically competent BL21 Star (DE3) One Shot *E. coli* cells and grown overnight at 37° C. 2 ml of an over-night culture were used to innoculate 100 ml of LB, 30 µg/ml kanamycin, and the cultures incubated at 37° C, 220 rpm until the cell density reached an optical density of 0.6 (approximately 3 hours). Expression of the recombinant protein was then induced with IPTG (1mM) for 5 hours.

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Bacteria were harvested by centrifugation at 4500 rpm for 10 minutes and the pellets lysed in lysis buffer (10 ml Bugbuster (Novagen), 10 µl Benzonase (Novagen), 0.4 µl lysozyme (Novagen) and 100 µl 1M imadazole for 20 minutes at room temperature. Cells were then spun down at 16000g for 20' at 4° C and the supernatant, containing soluble recombinant protein, removed to a clean tube.

Supernatant was added to prewashed Ni-Nta resin at a concentration of 5-10 mg protein per ml of resin and allowed to bind for 1 hour at 4° C. Protein-resin mix was then poured into a column, washed twice in 4 ml of wash buffer (2.5 ml 1M phosphate buffer pH8, 6.25 ml 4M NaCl, 1 ml 1M Imidazole pH8, 0.5 ml 10% Tween 20; made up to 50 mls in n.H₂O) and then eluted in 4x 0.5 ml fractions with elution buffer (250 µl 1M Phosphate Buffer pH8, 625 µl 4M NaCl, 1.25 ml 1M Imidazole pH8, 50 µl 10% Tween 20, Made up to 5 mls in n.H₂O). Fractions containing purified protein were detected by SDS-Page and Western blotting using an S-tag HRP conjugate (Novagen). Fractions containing purified recombinant protein were concentrated using YM10 columns (Millipore)

Figure 3A shows the induction of recombinant 2031 OR expression by IPTG over 24 hours. Protein samples were taken at time points, run on an SDS-PAGE gel and stained with coomassie. By 1 hr a band of the correct size was clearly induced compared to the uninduced samples. The amount of protein increased with longer induction times. Figure 3B shows a coomassie stained gel of the purified recombinant 2031 OR. Alternative expression systems can be used for expression in bacteria, such as the glutathione S-transferase or mannose-binding fusion-protein system.

Recombinant fragments of other 2031 ORs can be generated using the primer pairs and templates described in Table VIII, or similar primers and other 2031 OR listed in Table III.

5 Table VIII. Primer pairs for the recombinant expression of 2031 OR family proteins

Species		Template	Primer A	Primer B
A. fumigatus	•	SEQ ID No. 2	SEQ ID No. 55	SEQ ID No. 56
A. fumigatus	· · · · · · · · · · · · · · · · · · ·	SEQ ID No. 5	SEQ ID No. 57	SEQ ID No. 58
A. fumigatus		SEQ ID No. 7	SEQ ID No. 59	SEQ ID No. 60
A. nidulans		SEQ ID No. 9	SEQ ID No. 61	SEQ ID No. 62
C. ablicans	-	SEQ ID No. 11	SEQ ID No. 63	SEQ ID No. 64
M. grisea		SEQ ID No. 21	SEQ ID No. 65	SEQ ID No. 66

Example 7. Oxidoreductase assay and inhibitor screening

7.1 Oxidoreductase assay

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The assay for 2031 OR is based on methods described by Abramovitz & Massey (1976, J. Biol. Chem. 251: 5321-5326) and Stott et al. (1993, J. Biol. Chem. 268: 6097-6106) and is based upon the ability of this enzyme to oxidise the pyridine nucleotides NADH and/or NADPH. The peak of absorbance for the reduced form of these cofactors (i.e. NADH and NADPH) is at a wavelength of 340 nm whereas the oxidised forms of the cofactors (i.e. NAD+ and NADP+) do not absorb at this wavelength. Conversion of NAD(P)H to NAD(P)+ can therefore be monitored spectrophotometrically at a wavelength of 340 nm. A similar assay can be employed for all oxidoreductases that use NADH or NADPH as a cofactor.

Assays were carried out in 96-well plates. To each well was added the following;

Recombinant 2031 OR (10-1000 ng); 40 µl of 125-2500 µM NADPH; 1 µL 100 mM cyclohexeneone or other substrate, and the volume made up to 200 µL with 0.1 M potassium phosphate pH 7.0. Samples were incubated at room temperature and absorbance measurements were taken at 340 nm every 30 seconds for 10 min. The change in absorbance was expressed as nmoles NADPH oxidised, using the molar

extinction coefficient of NADPH and NADH at 340nm of 6270 (i.e., a 1M solution has an optical density of 6270 at this wavelength).

Initial experiments with a variety of potential substrates for recombinant 2031 OR showed that the protein had a functional dehydrogenase activity and determined that cyclohexenone was a better substrate than menadione, duroquinone or Nethylmaleimide. This is illustrated in figure 5. Final concentrations in the assay were as follows: $500 \, \mu M$ substrate, $1 \, \mu g/200 \, \mu L$ 2031 OR, $120 \, \mu M$ NADPH.

Although the physiological substrates of 2031 OR remain to be determined, generic oxidoreductase substrates such as ferricyanide, methylene blue, phenazine methosulphate and 2,6-dichlorophenolindophenol may also be used to assay for oxidoreductase activity.

Screens for inhibitors of 2031 OR can be carried out using the assay described above modified by the addition of putative inhibitor substances to the reactions and decreasing the amount of potassium phosphate buffer. Assays can be carried out in 384- or 1536-well plates to increase throughput of the screen.

7.2 High-throughput screen for the identification of 2031 OR inhibitors

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2031 OR inhibitors were identified by means of a high-throughput screen. The following reagents were prepared:

Assay plates: Compounds to be tested were dissolved in 100% DMSO (polypropylene vessels), diluted in water and loaded into 384 square well polystyrene plates (10µl/well). The final DMSO concentration in all assay wells was 5%v/v.

BNADPH (tetrasodium salt)/2-cyclohexen-1-one reagent; Solutions of NADPH (1.2917 mM in 100 mM potassium phosphate buffer, pH7.0) and 2-cyclohexen-1-one (10 mM in 100 mM potassium phosphate buffer, pH7.0) were prepared on the day of the assay and combined in a ratio of 1 part of 2-cyclohexen-1-one solution to 9 parts NADPH solution. Final assay well concentrations for NADPH and 2-cyclohexen-1-one were 465 μ M and 400 μ M respectively.

2031 OR enzyme: Recombinant enzyme was prepared as described in Example 6 and desalted as follows: 2.5 ml of eluted protein was loaded onto on to a PD10 column (Amersham) equilibrated with 25 ml of 0.1 M KPO₄ pH7. The protein was then eluted with 3.5 ml of 0.1 M KPO₄ pH7. Aliquots of the protein were stored at -80°C. For the

iscreen, protein was typically diluted to 5 to 11.25 μ g/ml in 100 mM potassium phosphate buffer, pH7.0.

Stop reagent: 0.4 M NaOH in water.

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The Km for 2-cyclohexen-1-one, the substrate for 2031 OR in the screening assay, was determined to be 100 μM. To give an increased signal, the screen was carried out using 2-cyclohexen-1-one at 4 times Km. The kinetics of the screen over the prescribed incubation time were such that reaction progress curves were both linear with time and protein concentration. The Z' value for the screen was equal to 0.77 and thus fully acceptable (Zhang et al., 1999, J. Biomolecular Screening, 4, 67-73). Consistency of signal between wells on plates, plate to plate and screen run to screen run were also acceptable for an HTS regime.

Assays were carried out using Tecan Freedom, Tecan TeMo and PerkinElmer Minitrak robots together with a ThermoLabsystems multidrop 384 and a Tecan Safire automated plate reader. 20 μl of enzyme followed by 20 μl NADPH/2-cyclohexen-1one solution were added to wells of the microtitre plates containing test compounds. 20 μl of 100 mM potassium phosphate buffer, pH7.0 was used for a duplicate set of plates for background no-enzyme controls; DMSO (diluted in the same way as solubilised compound stocks) was used for no-compound controls. Plates were incubated at room temperature for 30 minutes after which 25 μl of 0.4 M NaOH stop reagent was added. Plates were read at 340 nm on a Tecan Safire plate reader and data processed using 'inhouse' created Excel spreadsheets to convert raw data into percent inhibiton data. Secondary screens were carried out to measure dose response data for selected compounds, using essentially the same protocol as the pimary screen. The secondary screen used the Excelfit version 3 software (IDBS), with sigmoidal model 606, to graph appropriate inhibition values and determine IC50 data for compounds tested. Figure 6 shows typical results for 2 inhibitory compounds (A and B) identified by the primary screen and then assayed in the secondary screen.

Identification of the correct stop reagent for the HTS assay was not trivial. Initially, a chemical inhibitor of the system was sought to terminate the reactions in a pH independent manner, but it was found that NaOH offered more benefits than originally anticipated, in that it not only overcame the buffering in the reaction to fully

terminate the reaction, but also afforded a much greater protection for un-reacted NADPH. It is known that high levels of NaOH convert NADP, a product of the reaction which does not absorb at 340 nm, to a fluorescent product, which would interfere with the 340 nm readings taken (Passonneau and Lowry, 1993, Enzymatic analysis, a practical guide, pp.3-21 and p381. 1993 The Humana Press Inc. NJ USA.). Therefore, the NaOH level used in the HTS assay was chosen such that the amount of fluorescence from NADP conversion was reduced to an insignificant level, whilst fully terminating the reaction. The greater stability of the NADPH afforded by the use of NaOH meant that instead of immediate plate readings, plates could be read up to at least 20 hours post reaction termination (no further extended time points were investigated). This was an obvious advantage in that larger screens could be run. Plates stored for spectrophotmetric reading were sealed with self adhesive film and stored in the dark.

Example 8. Method for detecting fungal infection

The sequences described in the invention were exploited to diagnose fungal infections. Samples from patients potentially carrying an infection with A. fumigatus, A. nidulans, or C. albicans or rice leaves or stem potentially infected with M. grisea, or of alfalfa infected with C. trifolii, or wheat infected with F. graminearum, F. sporotrichioides, or M. graminicola, or other organisms, are processed to extract DNA using the DNAeasy Tissue kit or QIAamp DNA Blood Mini kit (Quiagen, Crawley, UK), although other DNA preparation methods are available and suitable. Once DNA has been prepared, PCR reactions are set up as follows:

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Reaction mix:

12.5 µl 2x ReddyMix PCR mastermix (ABgene)

- 1 µl primer A (5 pmol)
- 1 μl primer B (5 pmol)
- 30 5 μl template DNA
 - 5.5 µl nuclease-free water

Suiable primer pairs are given in the table IX below:

Table IX. Primer pairs for PCRs to diagnose fungal infection.

Species	Template	Primer A ¹	Primer B
A. fumigatus	SEQ ID No. 1	SEQ ID No. 67 (94)	SEQ ID No. 68 (286)
A. fumigatus	SEQ ID No. 4	SEQ ID No. 69 (239)	SEQ ID No. 70 (450)
A. fumigatus	SEQ ID No. 7	SEQ ID No. 71 (1097)	SEQ ID No. 72 (1271)
C. ablicans	SEQ ID No. 11	SEQ ID No. 73 (103)	SEQ ID No. 74 (277)
M. grisea	SEQ ID No. 20	SEQ ID No. 75 (385)	SEQ ID No. 76 (620)

Figures in brackets after SEQ ID No. indicate the base in the template at which the primer starts.

Appropriate controls include; (i) template DNA but no primers; primers but no template (negative controls); (ii) cDNA encoding fungal 2031 OR or DNA from cultured fungi instead of patient DNA (positive control).

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PCR reactions are run as follows:

	Step1	95°C	5 min
	Step2	95°C	1 min
	Step3	53°C	1 min 30sec
15	Step4	- 72°C	1 min 30sec
	Step5	72°C	10 min
	Step6	4°C	Hold

30 cycles of steps 2-4 are carried out and the PCR products examined on agarose gels. The production of a band of the correct molecular weight is diagnostic of the presence of the particular fungus. It may be additionally necessary to carry out diagnostic restriction digests of the PCR products. If necessary, PCR products are subcloned into a vector, such as pGEM-Teasy (Promega), and sequenced to verify that the PCR products are from the appropriate fungus.

Alternatively, the presence of an infection with A. fumigatus, A. nidulans, C. albicans or M. grisea, C. trifolii, F. graminearum, F. sporotrichioides or M. graminicola, or other organisms is detected by means of antibodies raised against the

fungal protein. One suitable means is the use of a capture ELISA. Here, microtitre plates are coated with a monoclonal antibody raised against the fungal protein. Then the plates are incubated with diluted patient samples, or appropriate protein extracts of samples (particularly if the samples are biopsies or plant tissues). Plates are then incubated with a polyclonal antibody (again against the fungal protein). Finally, binding of the second antibody was detected by means of an enzyme-coupled or fluorescently-labelled antibody directed against the polyclonal. In practise, two monoclonal or polyclonal antibodies or various combinations may be used.

10 Example 9. Production of an antibody

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Antibodies against the fungal 2031 ORs will be of considerable use as diagnostic reagents (see example 8 above). As an immunogen, recombinant domains are used (as described in Example 6). Alternatively, synthetic proteins encoding regions either unique to the individual 2031 ORs, or likely to provide cross-reactivity within a set of ORs, a set of species, or a range of genera are used. Peptides may need to be conjugated to carrier proteins before immunization.

Preimmune sera from animals to be immunised are screened against the immunogen to ensure that there is no endogenous cross reactivity. Animals (typically sheep, rabbits or mice) are then immunised. For polyclonal antibody production, the resulting sera is affinity purified using the immunogen cross-linked to a chromatography matrix. Alternatively, purification of the antibody fraction from the serum, e.g. using protein G or protein A cross-linked to a matrix, may be sufficient. Monoclonal antibody production proceeded by methods familiar to those skilled in the art.

The specificities of the resulting polyclonal and/or monoclonal antibodies are checked by ELISA and/or western blotting using the immunogen, related constructs or whole cell lysates and extracts as targets. Negative controls, such as other ORs, different constructs or different species are also employed to test specificity and/or to determine the range of species and/or genus cross-reactivity.

Example 10. Production of fungi with 2031 OR genes functionally disabled.

A BAC (bacterial artificial chromosome) clone library containing the A. fumigatus genome, partially digested with BamHI and inserted into the vector pBACe3.6 was purchased from the Sanger Centre, Cambridge, UK. The BAC clone containing the gene to be inactivated is identified by bioinformatics (BLAST searching of Sanger BAC and related databases) and the glycerol stock of the clone grown up in 50 ml LB, 20 µg/ml chloramphenicol at 37°C overnight. The overnight culture is centrifuged at 4,500 rpm for 15 min. The bacterial pellet is resuspended in 4 ml of Buffer P1 (Qiagen plasmid miniprep kit) and then 4 ml of buffer P2 (Qiagen plasmid miniprep kit, lysis buffer) is added and mixed gently by inverting 3-6 times. Proteins and genomic DNA are precipitated by adding 4 ml of buffer P3 (Qiagen plasmid miniprep kit, neutralizing buffer) and incubating on ice for 10 minutes. Following the centrifugation of the mixture at 4500 rpm for 30 min, the supernatant is transferred into a 50 ml falcon tube, an equal volume of phenol/chlorophorm (1:1) mixture is added, and the mixture centrifuged for 15 min at 4500 rpm. The supernatant is then transferred into an Oakridge tube and 0.7 volumes isopropanol are added. After mixing, the tube is centrifuged at 10,000 rpm (Beckman centrifuge, rotor JA-17) for 30 min at 4°C. The resulting pellet is washed with 2 ml 70% ethanol at the same speed. The resulting BAC DNA is resuspended in 100 µl buffer EB.

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The transposition reaction is carried out as follows. 7 μl purified BAC, 1 μl transposon pZVK2 (an engineered plasmid the sequence of which is given as SEQ ID No. 81), containing the mosaic ends of pMOD2 (Epicenter), a kanamycin resistance gene and a Zeocin resistance gene under the control of fungal promoter) and 1 μl EZ:TN transposase (Epicenter) are incubated at 37°C for two hrs after which 1 μl stop solution (1% SDS) is added and the mixture heated to 70°C for 10 minutes. Electrocompetent GeneHogs *E. coli* cells (Invitrogen) are then transformed with the transposed BAC, the cells plated onto LB agar, 25 μg/ml kanamycin, 20 μg/ml chloramphenicol, and plates incubated overnight at 37°C.

At least 96 colonies are picked and grown up in 96-well plates in 2xLB (double concentrated LB), 20 μ g/ml chloramphenicol, at 37°C overnight. BAC DNA is then purified using the Millipore montage 96 BAC KIT using a MWG ROBOSEQ 4200 robot. BACs containing the transposon inserted into the gene of interest are identified

by PCRs both spanning the gene of interest and extending from the transposon into the BAC. Insertion into the gene of interest is manifested as an increase in product size. Southern blots are also carried out to ensure that the transposon has only inserted once into the BAC.

The BAC is then linearised using a restriction enzyme determined to cut in the vector backbone but not the BAC DNA, and used to transform *A. fumigatus* strain Af293. *A. fumigatus* (haploid) protoplasts are prepared using 5% Glucanex (Novo Nordisk A/S) solution (in 0.6 M KCl) and shaking for 2 h at 80 rpm in 30°C. The protoplasts are washed with 0.6 M KCl and then with STC (Sorbitol, Tris, CaCl₂). The washed protoplasts are diluted in STC to 10⁵/ml and 100 μl transferred into 14 ml falcon tubes. 7 μl of linearised BAC are added to the tube and the whole mixture incubated on ice for 20 min. Transformation is carried out by adding 200 μl of PEG 8000 solution (60%w/v, pH 7.5) drop-wise over 2 min and then adding 800 μl PEG. The mixture is left at room temperature for 20 min. Transformed protoplasts are washed with STC, resuspended in 1 ml STC, spread onto CM-sorbitol- Zeocin (250 μg/ml) plates and incubated at 37°C.

After 4-10 days of incubation, zeocin resistant colonies are picked and checked for presence of the knocked-out gene by PCR using primers which specifically amplify the whole gene of interest. Usually 10-20 transformants are checked. The ectopic integration of the BAC gives two bands by PCR, one for the endogenous gene and one for the BAC/transposon construct, which has a higher molecular weight. Replacement of the endogenous gene with the transposon-modified gene results in a single band of higher molecular weigh by PCR. If none of the transformants show the disrupted endogenous gene, the gene of interest may be essential, with the knock-out cells having died and only cells where replacement is unsuccessful surviving. In this case, the transformation is carried out on diploids using the same method of transformation. Essentiality of the gene is then tested by rehaploidisation, and examining the segregation pattern in haploids.

Example 11. Rescue of MycoBank transformant with the 2031 oxidoreductase gene.

11.1 Preparation of the 2031 OR construct

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The 2031 OR gene with NheI overhangs was prepared by PCR using the primer pair; SEQ ID No 98 and SEQ ID No. 99.

PCR Reaction: 2.5 µl 10x PCR buffer

0.5 µl dNTPs

2 µl MgSO₄

1 μl forward primer (SEQ ID No. 98)

1 μl reverse primer (SEQ ID No. 99)

 $1~\mu l~gDNA$

Made up to 25 μl with n.H₂O

PCR Cycle: (1) 94° C, 5'; (2) 94° C, 1'; (3) 50° C, 1'; (4) 68° C 1'30s; (5) 68° C, 10'; (6) 8° C, Pause; Cycles 2 to 4 were repeated 40 times

The finished amplicon (~1260 bp) was run out on a 1% agarose gel, the appropriate band was cut out and purified using the Qiagen gel extraction kit and eluted off the column in 30 μl H₂O. The amplicon was ligated into pGEM Teasy using the following reaction mixture:

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5 μl 2x ligation buffer
1 μl pGEM Teasy vector

either 1, 2 or 3 μ l of insert

1 μl T4 DNA ligase

Reaction made up to 10 µl with n.H₂O

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The ligation reaction was incubated overnight in the fridge

2 μl of each ligation reaction was transformed by heatshock at 42°C into promega 96 select cells. After transformation, cells were incubated in SOC for 1 h at 37° C, 220 rpm. 50 and 150 μl aliquots were then spread over LB-Amp (100 μg/ml), IPTG-Xgal plates and left at 37° C overnight. Positive clones were identified by blue/white screening and were isolated and screened by PCR for correct insertion of

the 2031 OR insert using the above primers. Positive clones were sent away to MWG for sequence analysis.

11.2 Cloning of 2031 OR into the CbhB-Zeo vector

Plasmid DNA for 2031 OR in pGem Teasy (as described in 11.1) was digested overnight at 37° C with NheI. The 2031 OR insert fragment was then gel purified using the Qiagen gel extraction kit and ligated into CbhB-Zeo vector. This vector was constructed from pUC19 with the *A. fumigatus* CbhB promoter and terminator and the zeocin resistance gene.

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Ligation:

1 μl of T4 DNA ligase

1 μl of 10x ligase buffer

1 μl of CbhB vector (linearised and alkaline phoshatase treated)

1 μl of insert

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6 μl n.H₂O

Ligation reaction was left in the fridge overnight.

 $2~\mu ls$ of each ligation reaction was transformed by electroporation at 2.5 Kvolts, 200 Ω , 25 μF into Genehog cells. After transformation, cells were incubated in SOC for 1 h at 37° C, 220 rpm. 50 and 150 μl aliquots were then spread over LB-Amp (100 $\mu g/ml$) plates and left at 37° C overnight. Positive clones were isolated and screened by PCR for the correct insertion of the insert by PCR as above. Positives were sent to MWG for sequence analysis.

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11.3 Transformation into Mycobank mutant 2031

The CbhB-Zeo-2031 plasmid was digested with ScaI overnight at 37° C. Linearised plasmid was then run out on a 1% agarose gel and purified using the Qiagen gel extraction kit. Plasmid DNA was eluted in 30 μ ls of nH₂O.

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Mycobank mutant 2031 AF293 spores were swollen for 6 h at 37° C, 300 rpm, centrifuged 3500 rpm, 5' and resuspended in ice-cold nH₂O. Spores were spun

again, 3500 rpm, 5' then resuspended in 12.5 ml of YED medium and incubated for 1h at 30° C, 100 rpm. Spores were then counted and resuspend in EB buffer to a final concentration of $5x10^7$ spores per ml. 50 μ l of swollen spores were then transformed with 1-10 μ l of linearised CbhB-Zeo-2031 plasmid DNA at 1 Kvolt, 400 Ω , 25 μ F. Spores were transferred in to YED buffer and left for 90' at 37° C, 100 rpm. 100 and 200 μ l aliquots were then spread out on to CM-Zeocin (200 μ g/ml) plates and incubated at 37° C for 2-3 days.

Positive transformants on the CM-Zeo plates were picked into 5 ml of SAB broth and incubated overnight at 37° C, 220 rpm. Biomass was then filtered and collected on to Whatman paper. DNA was extracted using the Fast prep kit and cleaned up over a Qiagen miniprep DNA column. DNA was eluted off column in 30 μl of nH₂O.

PCR Screening was performed using the following primer sets:

Set A: Ox7race for (SEQ ID No. 51) + CbhBtR (SEQ ID No. 100)

15 Set B: Ox6race rev (SEQ ID No. 50) + CbhBpF (SEQ ID No. 101)

PCR Reaction: 12.5 µl 2x Reddy mix

1 µl each primer, from set A or B

1 μl plasmid DNA

20 Made up to 25 uL with water

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PCR Cycle: (1) 94° C, 5'; (2) 94° C, 1'; (3) 56° C, 1'; (4) 72° C 1'30s; (5) 72° C, 10'; (6) 8° C, Pause; Cycles (2) to (4) were repeated 40 times

Positive transformants which were demonstrated to have CbhB-Zeo-2031 in Mycobank mutant 2031 were put through the rehaploidation process to test their ability to grow on hygromycin compared with the untransformed mycobank mutant 2031. We found that the lethal 2031 phenotype was rescued by the insertion of the CbhB-Zeo-2031 plasmid, confirming the essentially of 2031 OR.

The reader's attention is directed to all papers and documents which are filed concurrently with or previous to this specification in connection with this application

and which are open to public inspection with this specification, and the contents of all such papers and documents are incorporated herein by reference.

All of the features disclosed in this specification (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive.

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Each feature disclosed in this specification (including any accompanying claims, abstract and drawings), may be replaced by alternative features serving the same, equivalent or similar purpose, unless expressly stated otherwise. Thus, unless expressly stated otherwise, each feature disclosed is one example only of a generic series of equivalent or similar features.

The invention is not restricted to the details of the foregoing embodiment(s). The invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed.

Sequence Listing

SEQ ID No 1

GTTCGACGTCATTGCCACGTTTCGACCCAAGGGCAGACGCCATGTCGCCGAGCGATCGCCGCGATATGCCTCGAATT 5 TGCGCCATTCGGCATCCAGTTTCCAGTGCCCTTCCCCGAATGACTGTCTCCACTATTCGGCAAGATTGTAAATCAAG CCTGAAGAAGCGGAGCAATTCTTGGAAGTCGTATGTTCTACTGATTTCTGTGCCTGGCGCAGACGGGTATATAAATA $\tt CCGATATCGACGTTCCTCCTGCCGAGGGCATCCCCTACTTCACTCCGGCCCAGAACCCTCCTGCCGGTACGGCAGCT$ AACCCCAGACCAATGGCCAGAAGATCCCCAAGCTCTTCACGCCCTTGACCATCCGTGGCGTCACCTTCCAGAACCG 10 CCTTGGTGTAAGTCCGTTTGCCCTTGCTCATATCGACGAAAGCTAATCCCCCGTCAGCTCGCGCCCCTCTGCCAATA TGCTGATTGAGGCGACCGCCGTCCAGCCCGAAGGCCGCATCACCCCTCAGGATGTCGGTCTGTGGAAGGACTCCCAG ATCGCCCCGATGCCCGGGTCATCGACTTCGTGCACAGCCAGGGCCAGAAGATCGGCGTGCAGCTTGCCCATGCCGG CCGGAAAGCCACCGTTGCGCCCTGGATCTCATTCTCGGCCATCGCGACGAGAAGGTCGGCGGATGGCCGGACC 15 GCGTCAAAGGGCCCGGCGATATCCCCTTTGCGGAGCCCTTCGCCAAGCCCAAGGCCATGACGCTGGATGAGATCGAG ${\tt CAGTTCAAGAAGGACTGGGGGGCCACGAAGCGCGCCATCGCCGGTGCGGACTTTGTCGAGATTCACAATGC}$ GCATGGATACCTGCTGTCGTCATTCCTCTCGCCGGCCGCCAACAACCGCACGGACCAGTACGGCGGGTCGTTCGAGA ACCGCATCCGGCTGTCTCTCGAGATTGCGCAGTTGACTCGGGACGCCGTCGGCCCTCATGTGCCCGTTTTCCTGCGC ATTTCGGCCTCGGACTGCTGCGAGAGACCCTGCCGGAGCAGAGCTGGAAGTCGGAGGATACCGTGCGGTTCGCGCA 20 GGAGCTGGTCAAGCAGGGCGCCGTTGATCTGATCGATATCAGCAGCGGTGGTGTTCTCGCGCAGCAGAAGATCAAGT $\tt CCGGCCTTCCAGGTGCCTTTTGCCGTGGCCGTGAAGAAGGCCGTCGGCGAAGCTGCTGGTTGCCGCCGTG$ ${\tt GGTGCCATCACCAACGGCAAGCAGGCGAATCAGATTCTAGAGGAGCAGGATATCGACGTTGCGCTGGTTGGCCGTGG}$ GTTCCAGAAGGATCCCGGTCTGGCCTGGACGTTTGCTCAGCACCTCGGCGTCGAAATCTCCATGGCCAACCAGATCC GCTGGGGCTTCACCCGGCGTGGAGGCACCCCGTACATTGATCCTTCGGTGTACAAGCAGTCTATTTTCGATGTATAG 25 AGTATAGATAGAGTTGAAGATGATACCTCATAGACGATCAATGGACCCTTGCATATTATTTCTCGTCTCCTGCGTAT GTTCAAGGTATTCACAGTAGCTGCGTCCTCTTAAGTTTCTCCGTCATTCGTTCTATTCTACTCCAATCGCAACGCAT GGCGACCACGGATCGAATTTCTCCGTCGTTCGTATCTGATCAATATAAAAAGCGGGGAATGGCTTGACCCCG

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SEQ ID No 2 ACCCATCAATAACCATCCACAATCTCCTACAACAAAAATGACTGTCGCCGATATCGACGTTCCTCCTGCCGAGGGCA TCCCCTACTTCACTCCGGCCCAGAACCCTCCTGCCGGTACGGCAGCTAACCCCCAGACCAATGGCCAGAAGATCCCC 35 AAGCTCTTCACGCCCTTGACCATCCGTGGCGTCACCTTCCAGAACCGCCTTGGTCTCGCGCCCCTCTGCCAATACTC TGATTGAGGCGACCGCCGTCCAGCCCGAAGGCCGCATCACCCCTCAGGATGTCGGTCTGTGGAAGGACTCCCAGATC GAAAGCCACCACCGTTGCGCCCTGGATCTCATTCTCGGCCATCGCGACGGAAAGGTCGGCGGATGGCCGGACCCGC 40 GTCAAAGGGCCCGGCGATATCCCCTTTGCGGAGCCCTTCGCCAAGCCCAAGGCCATGACGCTGGATGAGATCGAGCA GTTCAAGAAGGACTGGGTGGCGGCCACGAAGCGCGCCATCGCCGCCGGTGCGGACTTTGTCGAGATTCACAATGCGC ATGGATACCTGCTGTCGTCATTCCTCTCGCCGGCCGCCAACAACCGCACGGACCAGTACGGCGGGTCGTTCGAGAAC CGCATCCGGCTGTCTCCGAGATTGCGCAGTTGACTCGGGACGCCGTCGGCCCTCATGTGCCCCGTTTTCCTGCGCAT TTCGGCCTCGGACTGCTGCGAGGAGACCCTGCCGGAGCAGAGCTGGAAGTCGGAGGATACCGTGCGGTTCGCGCAGG 45 AGCTGGTCAAGCAGGGCGCCGTTGATCTGATCGATATCAGCAGCGGTGGTGTTCTCGCGCAGCAGAAGATCAAGTCC GGCCCTGCCTTCCAGGTGCCTTTTGCCGTGGCCGTGAAGAAGGCCGTCGGCGACAAGCTGCTGGTTGCCGCCGTGGG TGCCATCACCAACGGCAAGCAGGCGAATCAGATTCTAGAGGAGCAGGATATCGACGTTGCGCTGGTTGGCCGTGGGT TCCAGAAGGATCCCGGTCTGGCCTGGACGTTTGCTCAGCACCTCGGCGTCGAAATCTCCATGGCCAACCAGATCCGC TGGGGCTTCACCCGGCGTGGAGGCACCCCGTACATTGATCCTTCGGTGTACAAGCAGTCTATTTTCGATGTATAGAG 50 TATAGATAGAGTTGAAGATGATACCTCATAGACGATCAATGGACCCTTGCATATTATTT

CGCAGAATGTCGATCTCTTCGCAAACTCTCGGTGTATAGGACGCTCAGCAACGATCAAGG

SEO ID No 3

MTVADIDVPPAEGIPYFTPAQNPPAGTAANPQTNGQKIPKLFTPLTIRGVTFQNRLGLAPLCQYSAQDGHMTDYHIA

HLGGIAQRGPGLMLIEATAVQPEGRITPQDVGLWKDSQIAPMRRVIDFVHSQGQKIGVQLAHAGRKATTVAPWISFS
AIATEKVGGWPDRVKGPGDIPFAEPFAKPKAMTLDEIEQFKKDWVAATKRAIAAGADFVEIHNAHGYLLSSFLSPAA
NNRTDQYGGSFENRIRLSLEIAQLTRDAVGPHVPVFLRISASDWCEETLPEQSWKSEDTVRFAQELVKQGAVDLIDI
SSGGVLAQQKİKSGPAFQVPFAVAVKKAVGDKLLVAAVGAITNGKQANQILEEQDIDVALVGRGFQKDPGLAWTFAQ
HLGVEISMANQIRWGFTRRGGTPYIDPSVYKQSIFDV

SEQ ID No 4

atgtcgcaacctgttgtgcctgacatcgagaacaaacccgcgccgggtatctcgtactttactccggcgcaagagccgctgctggcaccgctgctaatcctcagtctgatggatcggcacctcccaagctcttccggccgctttcggtgcggg

tggaacagccgcttacagggaatgataatgagtagctatcgccactctgccaatactcagccgacgatggacacatg actccctggcatatggcacatcttggagggattgcccagcgagggccaggattcttgatggtcgaggcaacagcagt cgaaccggaaggcaggatcaccccgcaggacctgggactatggaaagactcgcagattgagccattgagccgcgtga tcgagtttgtccacagtcagaaccagcttatcggcgtgcagatcgcacacgcaggtcgcaaggccagcaccgtcgcg 5 ccatggctctcggccaacgataccgcctccgagaagatgggcggctggccaggccgcgtcaaaggcccgacaaatgt gcccttcaccgttaagaaccctgtgccgaaggagatgaccaagcaggatatcgaggatctgaagaccgcctgggtgg ccgctgtcaaacgggctgttaaggccggagccgactttatcgagatccacaatgcgcatggctatcttctgatgtcg ttcctctcccctgcggtcaacacgagaacagacgagtacggaggcagttttgagaatcgcatccggctcagtctgga gategecaageteaceegegaaaatgtgeecaaggatatgeetgtetteetgegggteteegeeacegattggetgg 10 aggaggtgcagccgaacaagcccagctggcgaggcgtggacactgtccgatttgcgaagatcctggcagaaacgggt tacgttgacgtgcttgacgtgagcagtggcggcactcattcggagcagcatatccacgcgaagccaggcttccaggc accetttgctattgccgtcaagaacgccgtcggggacaaactcgcagtggcatcagtgggtatgattgccagcgcgc atttggccaattccttgttggagaaggacggactggaccttgtgctggttggacgtggcttccagaagaacccgggg ctggtgtgggcgtgggccgacgagctgaatgtagagatctccatggctaatcagatccgatggggtttctcgcggcg 15 cggtgctggtccttacctcaggaagaaactcgagaagatataa

SEQ ID No 5 ATGTCGCAACCTGTTGTGCCTGACATCGAGAACAAACCCGCGCGGGTATCTCGTACTTTACTCCGGCGCAAGAGCC GCCTGCTGGCACCGCTGCTAATCCTCAGTCTGATGGATCGGCACCTCCCAAGCTCTTCCGGCCGCTTTCGGTGCGGG 20 GTCTGACCTTTCACAATCGCATTGGCCTATCGCCACTCTGCCAATACTCAGCCGACGATGGACACATGACTCCCTGG-CATATGGCACATCTTGGAGGGATTGCCCAGCGAGGGCCAGGATTCTTGATGGTCGAGGCAACAGCAGTCGAACCGGA AGGCAGGATCACCCCGCAGGACCTGGGACTATGGAAAGACTCGCAGATTGAGCCATTGAGCCGCGTGATCGAGTTTG TCCACAGTCAGAACCAGCTTATCGGCGTGCAGATCGCACACGCAGGTCGCAAGGCCAGCACCGTCGCGCCATGGCTC TCGGCCAACGATACCGCCTCCGAGAAGATGGGCGGCTGGCCAGGCCGCGTCAAAGGCCCGACAAATGTGCCCTTCAC 25 CGTTAAGAACCCTGTGCCGAAGGAGATGACCAAGCAGGATATCGAGGATCTGAAGACCGCCTGGGTGGCCGCTGTCA AACGGGCTGTTAAGGCCGGAGCCGACTTTATCGAGATCCACAATGCGCATGGCTATCTTCTGATGTCGTTCCTCTCC CCTGCGGTCAACACGAGAACAGACGAGTACGGAGGCAGTTTTGAGAATCGCATCCGGCTCAGTCTGGAGATCGCCAA GCTCACCCGCGAAAATGTGCCCAAGGATATGCCTGTCTTCCTGCGGGTCTCCGCCACCGATTGGCTGGAGGAGGTGC AGCCGAACAAGCCCAGCTGGCGAGGCGTGGACACTGTCCGATTTGCGAAGATCCTGGCAGAAACGGGTTACGTTGAC 30 GTGCTTGACGTGAGCAGTGGCGGCACTCATTCGGAGCAGCATATCCACGCGAAGCCAGGCTTCCAGGCACCCTTTGC TATTGCCGTCAAGAACGCCGTCGGGGACAAACTCGCAGTGGCATCAGTGGGTATGATTGCCAGCGCGCATTTGGCCA ${ t ATTCCTTGTTGGAGAAGGACGGACTGGACCTTGTGCTGGTTGGACGTGGCTTCCAGAAGAACCCGGGGCTGGTGTGG}$ GCGTGGGCCGACGAGCTGAATGTAGAGATCTCCATGGCTAATCAGATCCGATGGGGTTTCTCGCGGCGCGGTGCTGG 35 TCCTTACCTCAGGAAGAAACTCGAGAAGATATAA

SEQ ID NO 6
MSQPVVPDIENKPAPGISYFTPAQEPPAGTAANPQSDGSAPPKLFRPLSVRGLTFHNRIGLSPLCQYSADDGHMTPW
HMAHLGGIAQRGPGFLMVEATAVEPEGRITPQDLGLWKDSQIEPLSRVIEFVHSQNQLIGVQIAHAGRKASTVAPWL
SANDTASEKMGGWPGRVKGPTNVPFTVKNPVPKEMTKQDIEDLKTAWVAAVKRAVKAGADFIEIHNAHGYLLMSFLS
PAVNTRTDEYGGSFENRIRLSLEIAKLTRENVPKDMPVFLRVSATDWLEEVQPNKPSWRGVDTVRFAKILAETGYVD
VLDVSSGGTHSEQHIHAKPGFQAPFAIAVKNAVGDKLAVASVGMIASAHLANSLLEKDGLDLVLVGRGFQKNPGLVW
AWADELNVEISMANQIRWGFSRRGAGPYLRKKLEKI

45 SEQ ID No 7 ATGGGTTCCAACGCCTTCCGGTCCCCGCCGTCACCAAGTCCTCCTCCACCCCCTACTACACTCCCGCCAACAATGG AGGCGCCGCCCTGCACCCCGACGACCCCACGACCCCTACGCTCTTCCGGCCCTTACAAATCCGCAATGTGACGCTCA AGAACCGCATCATGGTGTCGCCCATGTGCATGTACTCCTGCGAGTCGGACCCGTCGTCTCCCCACGTCGGCGCCCTA ACAAACTACCACCTGGCGCATCTGGGCCACCTCGCCCTCAAAGGCGCAGGCCTCGTCTTCATCGAAGCCACCGCCGT GCAGCCCAACGGCCATCTCCCCCAACGACTCGGGCCTCTGGCAGGACGCCACCACCTCGGAACAATTCCTGGGGC 50 TGAAGCGGGTCGTCGAGTTCATGCACGCACAGGGCGCCAAGGTCGGGATCCAGCTTGCGCATGCGGGCCGGAAAGCG AGTGCCGTTGCGCCGTGGCTGGCGGCGCAGGCGGCAAGTCGAGTCTGAAGGCGGATGAGAGCGTTGGCGGGTGGCC CGCTGAGCACGGCCGAGGTCCGTCAGGTGGTGGCGGCGTTTGCGAAGAGCGCGCGGGTAGCGGTGCAGGCTGGGGTG 55 TGCGTACGGCGGAGCTTTGAGAACCGGACCCGGATCGTGCGCGAGGTTGCGGCGGCTATTCGTGCGGTGATTCCCG AGGGGATGCCCCTGTTTCTGCGTATCAGCGCCACGGAGTGGTTGGAGGGTCAGCCGGTGGCCGCGGAGTCGGCCAGC TGGGATATGCAGAGCTCGCTGGAGCTGGTCAAGAAGCTGCCCGAATGGGGCATTGACCTGGTGGATGTCAGCTCCGC CGCGAACCACAAGGACCAGAAGATCAACCTGCACACGGCCTACCAGACGGACCTGGCCGGGCAGATTCGCCAGGCCA ${ t TCCGAGCGGCTGGCGCTCGACTCTTGTGGGTGCTGTAGGTCTGATCACCGATTCGGAACAGGCGAGGGGACTAGTT}$ 60 CAGGGAGCGGACGAGCCAGCCGAGGCAATGCTGTCGGGACCTGAACCCAAGGCGGATGCCATTCTGATAGC ${\tt CCGTCAGTTCCTGCGCGAGCCAGAATGGGTGTTTTCCACGGCGAGAAAGTTGGGCGTGCCGGTGACTGTCCCGGTGC}$ AGTTTGGCAGGGCCATTTAG

MGSNAFRSPAVTKSSSTPYYTPANNGGAALHPDDPTTPTLFRPLQIRNVTLKNRIMVSPMCMYSCESDPSSPHVGAL
TNYHLAHLGHLALKGAGLVFIEATAVQPNGRISPNDSGLWQDGTTSEQFLGLKRVVEFMHAQGAKVGIQLAHAGRKA
SAVAPWLAAQAGKSSLKADESVGGWPADVVGPSGGEEHIFSPEEDAYWVPRALSTAEVRQVVAAFAKSARLAVQAGV
DVIEIHGAHGYLINEFLSPVTNKRTDAYGGSFENRTRIVREVAAAIRAVIPEGMPLFLRISATEWLEGQPVAAESGS
WDMQSSLELVKKLPEWGIDLVDVSSAANHKDQKINLHTAYQTDLAGQIRQAIRAAGASTLVGAVGLITDSEQARGLV
QGADEATAAEAMLSGPEPKADAILIARQFLREPEWVFSTARKLGVPVTVPVOFGRAI

SEQ ID No 9

5

10 ATGGCTCTCCCTGACGTCGAAAACACCCCCGCCGCCGCCGCCATCCCTACTTTACACCAGCACAGAACCCTCCTGCTGG AACAGCTGCCAACCCGCAAACCAGCGGCAATGCCGTCCCCAAGCTGTACACCCTCTGACGGTGCGTGGGGTGACCT TCCACAACAGACTTGGCCTCGCCGCTCTGCCAGTACTCCGCAGAAGACGGCCACATGACAGACTACCACATCGCG CACTTGGGAGGTATTGCCCAGCGCGCCCCGGTCTCATGATGATCGAGGCAACCTCCGTCTCACCTGAAGGCAGAAT $\tt CACGCCGCAGGACGTCGGTTTATGGAAGGACTCGCAGATTGCGCCCATGAAGCGCGTCATCGACTTCGTGCACTCGC$ 15 GGCATCGTCGCGACGGAGAAGGTCGGTGGCTGGCCGGATCGTGTGATCGGCCCGTCCACCGTGCCCTTCCACGAGAC TTTCCCCACCCCAAGGCCATGACCAAGGACGACATCGAGCAGTTCAAGCGCGACTGGTTTGATGCGTGCAAGCGGG 20 CCGTGACGCCGTCGGCCCCAACGTTCCTGTTTTTCTCGGTGTCTCCGCGACGGACTGGATCGAGGAGACCCTCCCCG AGGAATCGTGGAAGCTCTCTGACTCCGTCCGCTTCGCCGAAGCCCTCGCTGCCCAGGGCGCTATTGACCTGATCGAC GTCTCTTCCGGCGGTGTCCACGCCGCGCAGAAGATCAAGTCCGGGCCGGCTTTCCAGGCTCCCTTCGCTGTGGCTAT ${\tt CAAGAAGGCCGTTGGCGATAAGCTCCTTGTTGCGACGGTGGGCACGATCACGAACGGTAAGCAGCCGAACAAGCTGC}$ TTGAGGAGGAGGATTGGATGTTGCGCTTGTGGGACGTGGTTTCCAGAAGGATCCCGGTCTGGCGTCGGACTTTCGCG 25 ${ t CAGCATCTTGATGTTGAGATTGCGATGGCGAGTCAGATTCGGTGGGGATTCACAAGGCGCGGGGGCACGCCTTATAT$ CGACCCCAAAGCTTATAAGGAGAGCATCTTTGAGTAA

SEQ ID No 10

MALPDVENTPAAGIPYFTPAQNPPAGTAANPQTSGNAVPKLYTPLTVRGVTFHNRLGLAPLCQYSAEDGHMTDYHIA HLGGIAQRGPGLMMIEATSVSPEGRITPQDVGLWKDSQIAPMKRVIDFVHSQSQKIGVQIAHAGRKASNIAPWLMNK GIVATEKVGGWPDRVIGPSTVPFHETFPTPKAMTKDDIEQFKRDWFDACKRAIAAGADFIEIHNAHGYLLSSFLSPS SNTRTDEYGGSFENRIRLSLEIAQVTRDAVGPNVPVFLRVSATDWIEETLPEESWKLSDSVRFAEALAAQGAIDLID VSSGGVHAAQKIKSGPAFQAPFAVAIKKAVGDKLLVATVGTITNGKQANKLLEEEGLDVALVGRGFQKDPGLAWTFA QHLDVEIAMASQIRWGFTRRGGTPYIDPKAYKESIFE

SEQ ID No 11

ATGACAGTTCCATACCAAGTAAAACCATCAGATGAAATCAAAGGTGCTCCTGAGGTTTCCTATTACACTCCAGAACA 40 ${\tt GCCTGTTCCGGCTGGTACTTTTATCCCCAATCGTCAGATGAAGTTGCTCCCAAAATTTTTCAACCTTTAAAGATTG}$ GTAAGCTTGCTTTGCCAAACAGAATTGGGGTATCTCCAATGTGTCAATATTCTGCTGATTATAATTTTGAAGCAACT CCATACCATTTAATCCATTATGGTTCATTAGTGAATCGTGGGCCAGGTATCACCATTGTTGAAAGCACGGCTGTTTC TCCTGAGGGTGGATTATCACCTCATGATTTAGGAATCTGGAAGGATGAACAAGCAGAGAAATTGAAACCAATTGTCG ATTACGCTCATTCTCAAAAGCAATTAATTGCCATCCAATTGGGCCATGGTGGTAGAAAAGCTTCTGGTCAGCCCTTA 45 $\tt TTTTTGCACTTGGAACAAGTTGCAGATAAATCTGTCAATGGGTTTGCCGACAAAGCAGTTGCTCCTTCTGCATTGGC$ ${ t ATTCAGACCAAATGGTAATTTACCTGTTCCTAATGAGTTGACCAAAGATGAAATCAAACGTGTTGTTAAGGATTTTG$ GTGCTGCTGCTAGAAGAGCTGTTGAAATCAGTGGCTTTGATGCAGTTGAGATTCATGGTGCTCATGGTTATTTGATT ${\tt AATGAGTTCTATAGTCCTATTTCAAACAAGAGAACAGATGAATACGGTGGCAGTTTTGAAAATAGAACCAGATTTTT}$ AAAGGAAGTTATCGATAGTGTTAAATCAAGTATTCCAAACGATGTTCCAGTGTTTTTGAGAATCTCTGCTGCTGAAA 50 $\hbox{\tt ATAGTCCTGATCCAGAAGCTTGGACTATTGAAGATTCCAAAAAATTAGCTGACATTTTAGTAGAAAAGGGTATTGCT}$ $\tt TTGGTTGATGTTCATCTGGTGGTAACGATTATAGACAACCACCAAGATCTGGGATCAGTAAAGAGTTGAGAGAGCC$ AATCCATGTTCCGTTGTCTCGTGCAATTAAACAACATGTTGGTGACAAGTTATTGGTCAGTTGCGTTGGTGGGCCTTG AAAAAGATCCTGAATTGCTCAACAAATATTTAGAAGAAGGAACATTTGATCTTGCTTTGATCGGTAGAGGATTTTTA AGAAATCCAGGTTTGGTATGGGAGTTTGCCGATAAACTTGGTGTTAGACTCCACCAGGCCTTGCAGTTAGGTTGGGG

SEQ ID No 12

MTVPYQVKPSDEIKGAPEVSYYTPEQPVPAGTFYPQSSDEVAPKIFQPLKIGKLALPNRIGVSPMCQYSADYNFEAT
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FLHLEQVADKSVNGFADKAVAPSALAFRPNGNLPVPNELTKDEIKRVVKDFGAAARRAVEISGFDAVEIHGAHGYLI
NEFYSPISNKRTDEYGGSFENRTRFLKEVIDSVKSSIPNDVPVFLRISAAENSPDPEAWTIEDSKKLADILVEKGIA
LVDVSSGGNDYRQPPRSGISKELREPIHVPLSRAIKQHVGDKLLVSCVGGLEKDPELLNKYLEEGTFDLALIGRGFL
RNPGLVWEFADKLGVRLHQALQLGWGFWPNKQQIVDLIERTSKLEVN

TTTCTGGCCCAACAACAACAACTGTTGATTGATTGAAAGAACATCTAAATTAGAAGTAAATTAG

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SEQ ID No 13

ATGGAAAACAACAATACTATACCGGCATTATTTCAACCCATAAAGATCAGTGACTCGATCACATTACCTAATAGAAT TGGTGTTTCACCAATGTGCATGTATTCATCGTCACCAACTGACAATCAAGCCACTCTGTTTCATTTTGTTCATTATG 5 GATCATTTGCTGTACGTGGACCAGCATTAATCATTTTAGAGAGTATCTTTGTGTCCGAAAATTCCGGATTATCCATT CATGATTTAGGTCTTTGGAATGATGATCAAGCTCACAGTTTACGGAAAATTGTTGATTTATTCATGATCAAGACGG AATTTGCTGTATACAATTGAATCACGCTGGGCGAAAGATTGTTGAAGGGGGTACCATTCCAACAAATACAACATGGTT GGCAAGAACATTGTGTGGGGCCATCTACTGAGCCATTTAGTGATTCACACAATACACCACGAGAATTGACTGTTAAT GAAATAAATTCAATTGTGGAAGACTTTGCCAATGCAGCTTGGCGGGCTGTGGAAATCTCAAAATTCGATGCCATTGA 10 GCTCATTTGAAAACAGAGTTAGATTTCTTTTACAAATAATTGAGAATATAAAACGAAAGATAGAAACACCGATTTTC TTAAAGTTTCCAATGTCAGATAATTGTAGTGATCCGGAAGCGTGGTCTACGGAAGATGCATTGAAGTTGGCCGATCT TGTTATTGATTTAGGAGTAAAGGTGATCGACGTTACATCAGGTGGAAATGTTGCGCATTGCAAATCTAGATATCTAT TAAATGACGACAAACAACTACCTTCTCAAGTGCCCTTGGCTCGTAAATTGAAAAGCCACATTAGAAACCGATGTTTG 15 ATCGCATGCAGTGGAGGATTAGATCGAGACATATTTAAACTCGATGAGTTTATTGCTAATGGTGACTTTGATATAGC ${\tt ATTGATAGGTAAAGGATTTCTCAAAAACACTGGATTGATCAGCCGTATTGCTGACCAATTGCAAGCACAATTCAGAA}$ CAGCACCTCAATATAAGTTGGCCTTATCATAA

SEQ ID No 14

20
MENNNTIPALFQPIKISDSITLPNRIGVSPMCMYSSSPTDNQATLFHFVHYGSFAVRGPALIILESIFVSENSGLSI
HDLGLWNDDQAHSLRKIVDFIHDQDGICCIQLNHAGRKIVEGVPFQQIQHGWQEHCVGPSTEPFSDSHNTPRELTVN
EINSIVEDFANAAWRAVEISKFDAIEIHCANGCLIHQFLSKLTNKRADQYGGSFENRVRFLLQIIENIKRKIETPIF
LKFPMSDNCSDPEAWSTEDALKLADLVIDLGVKVIDVTSGGNVAHCKSRYLLNDDKQLPSQVPLARKLKSHIRNRCL
25
IACSGGLDRDIFKLDEFIANGDFDIALIGKGFLKNTGLISRIADQLQAQFRTAPQYKLALS

SEQ ID No 15

ATGGCCGACTTCACCCAGAAGAAGACCTCCTCCCCGCGGCCCCGGGTGTTCCCTTCTACACCCCGGCCCAGGTCCC 30 CGCCGCCGGCACTCCCCTCCACCCCCGGCGATGTCCCTACTCTCTTCACCCCTCTCAAGATCCGTGGTGTTG AGCTCCAGAACCGCTTCGCCGTTGCGCCCATGTGCACCTACTCTGCCGACGATGGCCACATGACCGACTGGCACCTT GTCCACCTGGGCTCCTTCGCCCTCCGCGGTGTCCCCCTCACCATCTTCGAGGCCACCGGCGTCCTCCCCAACGGCCG CATCACCCCGAGTGCTCTGGTCTCTGGCAGGACTCCCAGATTGCGCCCCTCAAGCGCATCGTCGACTACATCCACT 35 CAACGAGGAGACCTTCCCCTTCCCCAAGGAGATGACCGTCGAGCAGATCCACGAGCTCGTCGAGGCCTGGAAGGCGT $\tt CTGCCCAGCGTGCCCTCAAGGCCGGCTTCGACCTCATTGAGATCCACGCCGCCCACGGCTACCTCATTTCCGAGTTC$ TTGAGCCCCATCTCCAACCAGCGTACCGACCAGTACGGTGGCTCCTTCGAGAACCGCACCCGCGTTCTCCGCGAGAT 40 ACACCGGCCAGCCCTCGTGGGACCTCCAGCAGACCATTGAGCTCGCCAAGATCCTCCCCGACCTCGGCGTCGACCTC CTCGACGTCTCTCCGGCGGCAACAACAAGGACCAGAAGATCAACGTCCACACCTACTACCAGATCGACATGGCCGA ${\tt GCAGATCCGCGCGCCGTGCACGAGCCGGCAAGCAGCTCCTCGTCGGTGCCGTCGGCTTGGTCACCTCGGCTGAGA}$ TCGCCAAGGAGACCGTCCAGGAGAAGGAGGATGGCAGAGTCACCATCCAGCGCGAGAACGGCGCCAAGACTCGTGCC GATATGGTCCTTGTTGCCAGGCAGTTCTTGAAGGAGCCCGAGTTCGTCCTCACTGTCGCCGACGAGTTGGGTGTTGA 45 TGTCAAGGCCCCTGTTCAGTACCTCCGTGGTCCTCTTAGCAGCAGGCCCAAGAAGTTGACCACTGTTCCTTAA

SEQ ID No 16

MADFTQKKTSSPAAPGVPFYTPAQVPAAGTPLPSTPGDVPTLFTPLKIRGVELQNRFAVAPMCTYSADDGHMTDWHL

VHLGSFALRGVPLTIFEATGVLPNGRITPECSGLWQDSQIAPLKRIVDYIHSQGQKAGIQLAHAGRKASTKAPWHYQ
RGKSELAGPEQGGWPENVWAPSAISYNEETFPFPKEMTVEQIHELVEAWKASAQRALKAGFDLIEIHAAHGYLISEF
LSPISNQRTDQYGGSFENRTRVLREIISAVRSVIPEDMPLFVRVSATEWMEYTGQPSWDLQQTIELAKILPDLGVDL
LDVSSGGNNKDQKINVHTYYQIDMAEQIRAAVHEAGKQLLVGAVGLVTSAEIAKETVQEKEDGRVTIQRENGAKTRA
DMVLVARQFLKEPEFVLTVADELGVDVKAPVQYLRGPLSSRPKKLTTVP

SEQ ID No 17

15 SEQ ID No 18

ATGGCTACTTCCACTACCTCCGACCTCAAACTCTCCCCAACCCCTCACCCTCCCCAATGGCCTTACCCTCCCCAACCG CCTCGTCAAAGCCGCCATGGCCGAACAAATGGGCTTCGGCAACCACCTGCCCAACCCCGAACTCGCCGCCGTCTACG $\tt CCACCTGGGCCGGGGGGCTGATTCTCACCGGCAACGTCCAAGTCGACCACGCGCACAAGGGCGACGCC$ 20 CACGACATCAGCCCCAACCACCCCGGCACCACGCCCGAGCAGACCGTCACGGCCTTCAAGGCCTGGGCGGACGCCGC GCGCCTGAATGGCCAGTCCAAAACGCCTGTGGTCGTGCAGATCAACCACCCTGGTCGCCAGAGTCCGATGGGCGCGG GCACGCGGGGACTGTGGGAGAAGGCGGTGCCGCCCCCGGTGCCGTTGGTGTTTGGGAGAGGCGTTTGTGCCTCGC TTGTTGTCGAAAGTGCTTTTCGGCACGCCGCGGAGCTGACGGTTGCGGAGATCAAGGATATCGTGCAAAAGTTTGC GGTGÄCGGCGAGGATCACGGCCGAGGCCGGGTTCAATGGCGTGGAGATCCATGCGGCGCATGGATACCTGTTGGCGC 25 AGTTCTTGAGCAAGAAGACAACAGGCGCGGGGATGAGTATGGCGGGTCGGCTGAGAACAGGGCGAGGATTGTTGGG GAGATTATTAAGGAGTGCAGGAGGCAGGTGACTGAGGCGGTGGAGAAGAGGGCGGAAGAAGTTTGTGGTGGGAAT CAAGCTGAACAGTGCGGATTGGCAGGCGGGACGCGATGGAAAGGAGGAGGAGGAGGACGGATACGGCGGAGGAGGTGT TGAAGCAGATTGAGCTTTTTGAGCAGTGGGGGATCGACTTTGTCGAGGTTAGCGGTTGCCAGTTATGAGGATCCTCAG ATGGCCAACGGTCCCAAGCCCGAAAAGTCCGAACGCACCATGGCCCGCGAGGCCTTCTTCCTCGAGTTCGCCAAGAT 30 $\tt CATCCGCACCAAGCTTCCCCAAGCTTCCTCATGGTCACCGGCGGCTTCCGCACTCGTCAGGGCATGGAGGCCGCTT$ TGGAATCCGATGATTGCGACATGATCGGTATCGGACGCCCGGCCATCATCAACCCTTCGCTTCCCGCCAACTTGATC $\tt CTCAACCCGGAGGTGCCGGATGCCCGCTTGTTCGACAAGAAGAGGGCTGAGCCGCACTGGATCGTTGAGAA$ TTTAG

SEQ ID No 19

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MATSTTSDLKLSQPLTLPNGLTLPNRLVKAAMAEQMGFGNHLPNPELAAVYATWARGDWGLILTGNVQVDHAHKGDA
40 HDISPNHPGTTPEQTVTAFKAWADAARLNGQSKTPVVVQINHPGRQSPMGAGTRGLWEKAVAPSPVPLVLGEAFVPR
LLSKVLFGTPRELTVAEIKDIVQKFAVTARITAEAGFNGVEIHAAHGYLLAQFLSKKTNRRGDEYGGSAENRARIVG
EIIKECRRQVTEAVGEEEAKKFVVGIKLNSADWQAGRDGKEEEETDTAEEVLKQIELFEQWGIDFVEVSGGSYEDPQ
MANGPKPEKSERTMAREAFFLEFAKIIRTKFPKLPLMVTGGFRTRQGMEAALESDDCDMIGIGRPAIINPSLPANLI
LNPEVPDADARLFDKKRAEPHWIVEKLGMKSIVGAGVEVTWYVSELKKLAKF

SEQ ID No 20

50 atgtcggcagaaaagaagttttgagcaaaccggccgcggggtgccttactacaccccagcccaggagccgccggc agggacccctttgcagcagcaggacgccatcccaacgctgttcaagcctctgaagatccgtggcgtcgagctctcca accgctttggcgtctcgcccatgtgcacctactcagccgacgatggccacctgaccgacttccacttggtgcacctg ggccagttcgccctgcacggcacggccctgaccattgtcgaggccacatccgtcacgcccaacggacgcatctcgcc cgaggacageggcctgtggcaagacagccagategctectetgegeegcategtegactaegtgcacagecagggee 55 aaaagategeeateeaaetggeteatgeeggeegeaaggeeageaeaaaggeeeeetggeaegaeteetteaeeeee agcggcgagtataagccgagagagggcttacaggtcgtcggacccgagtatggcggctggcctgatgacgtctgggc $\verb|cccgagcgccatcccgttctcggaggactttccgaaccccaaggagattgaccgttgaggagattgagggactcgtca|\\$ ccagctttgtggacgctgccaagcgtgccatcgaggccggcgtcgacattattgagattcacggcgctcacggttac ctgatcaccgagttcctttcgccgctatcaaacgtaagtggagatactttgtgtggggctgtgcgcatactccctcg 60 cacccgggtcctgatcgatattatcaaggccgtccgggcagtgattcccgaggagatgccactcttcgtccgaatct ccgcgaccgaatggatggagtacgccggcgagcctagctgggacctcgagcagagcacacagcttgccaagctcctc ccggacctgggtgtcgacctgctcgacgtcagctcgggcggaaactcggtggcccaaaagatcgagctcacgccgta ctaccagatcgacctggcagccaagatccgcgaggccgtcggcgataggttgctcataggcgcggtcggcaacatca 65 acacggctgacattgcgcgcgatgtcgtggatgagcagggcgccgagaaggtggccgaggccaagcagacgcatgac

accatcgaggtcgtgagcgaatcacatggcggcaagaccaaggcggatctggtcctcattgctcgccagttcctgcgcgggcctgagtttgtgctgaggacggcgcataaccttggggtcaatgtgcagtggcctcaccaataccacagagcagtggcgcaagggtgcaaggatttga

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SEQ ID No 21

ATGTCGGCAGAAAGAAGACTTTGAGCAAACCGGCCGCCGGGGTGCCTTACTACACCCCAGCCCAGGAGCCGCCGGC AGGGACCCCTTTGCAGCAGCAGGACGCCATCCCAACGCTGTTCAAGCCTCTGAAGATCCGTGGCGTCGAGCTCTCCA 10 ACCGCTTTGGCGTCTCGCCCATGTGCACCTACTCAGCCGACGATGGCCACCTGACCGACTTCCACTTGGTGCACCTG GGCCAGTTCGCCCTGCACGGCACGGCCCTGACCATTGTCGAGGCCACATCCGTCACGCCCAACGGACGCATCTCGCC AAAAGATCGCCATCCAACTGGCTCATGCCGGCCGCAAGGCCACAAAGGCCCCCTGGCACGACTCCTTCACCCCC AGCGGCGAGTATAAGCCGAGAGAGGGCTTACAGGTCGTCGGACCCGAGTATGGCGGCTGGCCTGATGACGTCTGGGC 15 $\tt CCCGAGCGCCATCCCGTTCTCGGAGGACTTTCCGAACCCCAAGGAGATGACCGTTGAGGAGATTGAGGGACTCGTCA$ $\tt CCAGCTTTGTGGACGCTGCCAAGCGTGCCATCGAGGCCGGCGTCGACATTATTGAGATTCACGGCGCTCACGGTTAC$ GGTCCTGATCGATATTATCAAGGCCGTCCGGGCAGTGATTCCCGAGGAGATGCCACTCTTCGTCCGAATCTCCGCGA CCGAATGGATGGAGTACGCCGGCGAGCCTAGCTGGGACCTCGAGCAGACCACACCTTGCCAAGCTCCTCCCGGAC 20 $\tt CTGGGTGTCGACCTCGACGTCAGCTCGGGCGGAAACTCGGTGGCCCAAAAGATCGAGCTCACGCCGTACTACCA$ GATCGACCTGGCAGCCAAGATCCGCGAGGCCGTCGGCGATAGGTTGCTCATAGGCGCGGTCGGCAACATCAACACGG CTGACATTGCGCGCGATGTCGTGGATGAGCAGGGCGCCGAGAAGGTGGCCGAGGCCAAGCAGACGCATGACACCATC GAGGTCGTGAGCGAATCACATGGCGGCAAGACCAAGGCGGATCTGGTCCTCATTGCTCGCCAGTTCCTGCGCGAGCC TGAGTTTGTGCTGAGGACGGCGCATAACCTTGGGGTCAATGTGCAGTGGCCTCACCAATACCACAGAGCAGTGTGGC 25 GCAAGGGTGCAAGGATTTGA

SEQ ID No 22

MSAEKKTLSKPAAGVPYYTPAQEPPAGTPLQQQDAIPTLFKPLKIRGVELSNRFGVSPMCTYSADDGHLTDFHLVHL
GQFALHGTALTIVEATSVTPNGRISPEDSGLWQDSQIAPLRRIVDYVHSQGQKIAIQLAHAGRKASTKAPWHDSFTP
SGEYKPREGLQVVGPEYGGWPDDVWAPSAIPFSEDFPNPKEMTVEEIEGLVTSFVDAAKRAIEAGVDIIEIHGAHGY
LITEFLSPLSNKRTDKYGGSFENRTRVLIDIIKAVRAVIPEEMPLFVRISATEWMEYAGEPSWDLEQSTQLAKLLPD
LGVDLLDVSSGGNSVAQKIELTPYYQIDLAAKIREAVGDRLLIGAVGNINTADIARDVVDEQGAEKVAEAKQTHDTI
EVVSESHGGKTKADLVLIARQFLREPEFVLRTAHNLGVNVQWPHQYHRAVWRKGARI

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SEQ ID No 23

ATGACTATTGTTAATGAAGGAGCCGAAAATGTTGGTTATTTTACACCTGCGCAAAAAATACCAGCTGGAGCGGCGAT 40 CGATGTGCACTTATTCCGCTGACCAAGAAGGGCATTTGACAGATTTTCACCTAGTACATCTTGGAGCGATGGGAATG $\tt CGTGGCCTGGCCTTGTAATGGTAGAAGCGACAGCGGTTTCCCCAGAGGGACGAATTTCACCTAATGATTCAGGATT$ ${\tt ATGGATGGAGTCGCAAATGAAGCCGTTACGAAGAATTGTTGAATTTGCTCATTCGCAAAATCAAAAAATTGGGATTC}$ AATTGGCGCATGCTGGTAGAAAGGCTAGCACCACTGCTCCTTATCGAGGATACACAGTTGCGACTGAAGCTCAAGGT 45 GGGTGGGAGAATGATGTTTATGGACCAAATGAAGACAGGTGGGACGAAAACCACGCTCAACCTCATAAGTTAACTGA AAAGCAATATGATGAATTAGTGGATAAGTTTGTTGTTGCTGCGAAGCGTGCAGTTGAAATAGGTTTTGATGTAATTG AAATTCATGGCGCTCATGGTTATCTTATATCGTCAACAGTTAGTCCTGCCACTAATGACCGCAATGACAAGTATGGT GGGACATTTGAGAAACGTATTTTGTTTCCTATGGAAGTTGTCCATTCTGTTCGTAAAGCAATTCCAGATAGTATGCC CTTGTTTTATAGAGTAACGGCTACAGATTGGTTGCCCAAAGGACAAGGATGGGAGATAGAAGATACAGTTGCATTAG 50 CAGCGAGGCTTCGCGATGGTGGTGTTGACTTGATAGATGTTAGCTCTGGTGGTAATCACAAGGATCAAAGAATTGAG GTGAAGGATTGCTATCAAGTTCCTTTTGCGGAAAAGATTAAGGATCAAGTGAATGGAATACTACTTGGCGCTGTCGG AATGATCAGGGATGGTCTTACGGCGAATGAAATCCTAGAAAGTGGAAAAGCTGATGTTACTTTTGTCGCAAGGGAGT TCTTAAGGAACCCGTCGTTGGTGCTAGACAGCGCGAACCAGTTGGGTGAAAATGTTGCATGGCCAGTTCAGTATGAC TATGCAGTTAAGGGACACAGAAAGTTACGTTGA 55

SEQ ID No 24

MTIVNEGAENVGYFTPAQKIPAGAAIGVPQTKLFTPLKIRGVEFHFTNRMFVSPMCTYSADQEGHLTDFHLVHLGAM
GMRGPGLVMVEATAVSPEGRISPNDSGLWFTMESQMKPLRRIVEFAHSQNQKIGIQLAHAGRKASTTAPYRGYTVAT
EAQGGWENDVYGPFTNEDRWDENHAQPHKLTEKQYDELVDKFVVAAKRAVEIGFDVIEIHGAHGYLISSTVSPAFTT
NDRNDKYGGTFEKRILFPMEVVHSVRKAIPDSMPLFYRVTATDWLPKGQGWEIEDTVAFTLAARLRDGGVDLIDVSS
GGNHKDQRIEVKDCYQVPFAEKIKDQVNGILLGAVGMIRDGLFTTANEILESGKADVTFVAREFLRNPSLVLDSANQ
LGENVAWPVQYDYAVKGHRKLR

65 SEQ ID No 25

15 SEQ ID No 26

ATGACGGCACCAGGACAAGGCCGCCCCGGTGTGCCGTTTTACACCCCGGCCCAGGAGCCTCCCGCGGGAACGCC
AGTCGACGCCAGCACGCTCCTCAAGCCCCTCCGCATCCGCGACCTCACCATCAACAACCGCATCTGGG
TCAGCCCCATGTGCCAGTACTCCGCCGACAATGGCCACCGCGCCGACTACCACCTCGTCCACCTGGGCCAGTTCGCC
CTGCACGGCGCCCGCCTGTCCATGGTCGAGGCCACCGCCGTCGAGGCTCGTCGCCGATCTCCCCCGAGGATGTCGG
TTTGTGGCAGGACTCGCAGATTGCGCCGCTGAAGCGCATCGTCGACTTTATCCACTCGCAGAACCAGGTCGCGCCA
TCCAGCTCGCCCACGCCGGTCGCAAGGCTAGCACCCTGGCACCGTGGATCACCGAGGCTCGCGCCAAGGCCTGGCT
CAGGAGAGCGAGAACGGCTGGCCCGACGACGTTGTGGCTCCCAGCGCGATTCCTTACACCAAGGACTGGGCCACACC
GCGTGAGTTGACTACCGAGGGTCGAGGGTCTGGGTGAAGAAGTTCGCCGAGTCGGCCAAGAGGTCAAATCGAGCTG

25
GTTTTGACGTCATTGAGATCCACGCCGCT

SEQ ID No 27

MTGTANKAAPGVPFYTPAQEPPAGTPVDASTAPTLFKPLRIRDLTINNRIWVSPMCQYSADNGHATDYHLVHLGQFA
LHGAALSMVEATAVEARGRISPEDVGLWQDSQIAPLKRIVDFIHSQNQVAAIQLAHAGRKASTLAPWITEARGKALA
QESENGWPDDVVAPSAIPYTKDWATPRELTTEXSRVWVKKFAESAKRSNRAGFDVIEIHAA

SEO ID No 28

SEQ ID No 29

60 SEQ ID No 30

65 E

SEO ID No 31

SEQ ID No 32

25 SEQ ID No 33

30

 $\label{topiconstruction} TDEYGGSFENRIRVVLEILDLIRAAIPETTPVLVRVSATDWFEFDSQFKDEFPESWTVEQTCQLARILPKHGVDLVD VSSGGIHPKSAIAIKSGPAYQVDLAKQVKKAVGDSVLVSAVGGIKTGHLAEEVLQSGIDIVRAGRWFQQNPGLVRAF ANELGVEVKMANQIDWSFKGRGKKVNKSSL$

SEQ ID No 35

45 MPKCEANGHHKIIINKEAPNVPFYTPVQDPPAGTSYDVQPEGSLFSLIKIRNLTLQNRIFVSPMCQYSAKDGVMTPW HKQHLGSFAARGPGLIVTEVNAVSPEGRISPEDAGIYDDGQLGPLRDIVDFVHSQGAKIAIQIGHAGRKASTVVPWL DRKNTAF

SEQ ID No 36

CGAGGCCCGGGACTGTCCATGGTAGAGGCCACCGCTGTTCAAAACCACGGTCGCATCACGCCTCAGGACGTTGGTCT CTGGGAAGATGGACAAATCGAGCCCTTGAAGCGCATCACTACTTTTGCCCACAGCCAAAGCCAGAAGATTGGTATTC ${\tt AGCTCTCGCACGCTGGTCGTAAGGCTAGTTGTGTATCTCCGTGGTTGAGCATCAACGCTGTTGCCGCTAAGGAAGTC}$ ${\tt GGTGGCTGGCCAGACAACATTGTTGCTCCTTCTGCCATCGCACAAGAAGCTGGCGTGAACCCTGTTCCCAAGGCCTT}$ TCATCGAGATCCATGCAGCTCATGGATACKTGCTTCACCAGTTCTTGAGTCCAGTCAGTAACCAAAGAACCGATGAG TATGGTGGCAGCTTCGAGAACCGTATCAGAGTCGTCTTGGAGATCATTG

SEQ ID No 38

- 10 ARGIIDNIAAEGAPYYTPAQDXPAGTQTSGSTKVFTXITIRGVTFPNRLFLAPLCQYSAKDGYATDWHLTHLGGIIQ RGPGLSMVEATAVQNHGRITPQDVGLWEDGQIEPLKRITTFAHSQSQKIGIQLSHAGRKASCVSPWLSINAVAAKEV GGWPDNIVAPSAIAQEAGVNPVPKAFTKEDIEELKNDFLAAXKRAXRAGFDVIEIHAAHGYXLHQFLSPVSNQRTDE YGGSFENRIRVVLEII
- 15 SEQ ID No 39 CCTCAAGATCCGAGGTCTTACCCTCCAGAACCGTATTATGTTGAGGGGGCTCTGCCAGTACTCTGCTCCCGACGGAC ACTACACAATGTGGCATCACCCACATGGGCGGCATCATCCAACGCGGTCCCGGACTCACCTGCGTTGAAGCCACA $\tt GCCGTGACTCCTCAAGGTCGCATCACGCCTGAAGACGTCGGTATCTGGCAAGATTCTCAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCTGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCTGAGCCTCTTGCCAAGATCGAGCCTCTTGCCAAGATCGAGCCTCTTTGCCAAGATCGAGCCTCTTTGCCAAGATCTGAGCAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATATCTGAGATATCTGAGATCTGAGATATCTGAGATCTGAGATCTGAGATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATATCTGAGATATATCTGAGATATCTGAGATATATCTGAGATATATCTGAGATATATCTGAGATATATCTGAGATATATCTGAGATATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGATCTGAGA$ GGTCGTCGAGTTTGCCCACTCCCAGAACCAGAAGATCATGATTCAGTTGGCGCATGCGGGCCGCAAAGCGAGCACTG 20 TGGCACCATGGTTAAGCGGCGGCGATGTTGCTGGTGAGGACGTCAACGGATGGCCACAGGATGTCTGGGCGCCCAGT GCGATTCCATGGAACGAGAAGCACGCTGTCCCAAAGGAGATGTCGTTGGATGATATCGAGGCTTTCAAGAAGGCGTT

TGGAGAGGCGGTCAAGCGGGCATTGAAGGCTGGATTTGATGTTATTGAGATTÇACAATGCTCACGGATACCTCCTCC ACGAATTCATCTGCCTGAGAGCAACACCAGGACCGACAAGTACGGGCGGAAGCTGGGAAAACCGCACTCGTCTGACA

ATGGAAAGTCGTCGACCTTGTCCGCAGCATT

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SEO ID No

40LKIRGLTLQNRIMLRGLCQYSAPDGHYTMWHHTHMGGIIQRGPGLTCVEATAVTPQGRITPEDVGIWQDSQIEPL AKVVEFAHSQNQKIMIQLAHAGRKASTVAPWLSGGDVAGEDVNGWPQDVWAPSAIPWNEKHAVPKEMSLDDIEAFKK 30 AFGEAVKRALKAGFDVIEIHNAHGYLLHEFICLRATPGPTSTGGSWENRTRLTMESRRPCPQH

SEQ ID No 41

- 35 TTTACGTGGGATGAGAGGTCCTCGAGCGACCCTAGTGGAGGCTACTATGCGCCGAGAGAGTTGTCGGTCAGAGAGAT CAAGGAGATGGTCCAAGACTGGGCGACAGCAGCGAAAAGGGGCGGTGAAAGCGGGCGTGGATGTAATCGAAATCCACG GCGCGCATGGGTACCTCATCCACGAATTCCTCTCACCCATTACCAACCGCCGGACAGATTCTTACGGCGGTTCTTTC ${\tt GAAAACCGTACCCGTCTACTCATTGAAATCGTAACAGCCGTCCGAGCCGCGATGCCCTCCAGCATGCCTCTTCCT}$ $\tt CCGCCTCTCCTACAGAATGGAAGAAGATACCGACATCGGCAAGAAGTTCGGAAGCTGGGATGTCGAAAGCACGA$ TCAAGATCTCCAAAATCCTGGCCGACTTGGGCGTTGATCTCCTCGACGTGTCTTCCGGTGGGAATCATCCTCAGCAG 40 AAAATCAACATGTTCAACACC
- SEQ ID No 42 45 LPSKRAGKEAGGWPEDVVGPSGGEDFTWDERSSSDPSGGYYAPRELSVREIKEMVQDWATAAKRAVKAGVDVIEIHG AHGYLIHEFLSPITNRRTDSYGGSFENRTRLLIEIVTAVRAAMPSSMPLFLRLSSTEWMEDTDIGKKFGSWDVESTI KISKILADLGVDLLDVSSGGNHPQQKINMFNT

SEQ ID No.

50 ATGTCCCCACCACGCTTCGAAGCGGCCCCTGCCGACCCCTCACCGCTCGGCACGCCGCTCAAATACCCCGTCTCGGG $\tt GCGGTCGGCCCAACCGGTTCCTCAACGCGGCCATGTCGGAGGGCCTGGCGACGTTTGACGAGGCGGACCCGTCCA$ AGCGCGGCATCCCGACGGAGCAGCTGGTGCAGCTGTACCGGCGCTGGGGCCAGGGGCGAGTGGGGCCAGATCCAGACG GGCAACGTCATGATCGACCCGGAGCACCTCGAGGCCCCGGGCAACATGGTGGTGCCGCGCGACGCCGAGCCCTCGGG CGAGCGCTTCGACATGTTTTCCAAGCTCGCCGCCGCCGCCAAGGAGCACGGCAGCCTCATCGTCGCGCAGGTCGGAC ACCCCGGTCGCCAGGCCCGCGGCAGCGTCCAGCAGCACCCCATTAGCGCCAGCGACGTGCAGCTTAAGCAGGAGATG 55 TTTGGGTCAAAGTTTGGCGTGCCCAGGCCCGCTACCAAGGAGGATATTAAGGCGGTGATTGAGGGTTTTGCCCACAC GGCCGAGTACCTTGAAAAGGCCGGTTTCGACGGTATCGAATTGCACGCCGCCCACGGTTACCTGCTGGCCCAATTCC ${\tt ACGGCCGAGGTCCGCAGGCGAGCAAGAATTTCATCCTCGGCATCAAAATTAACAGCGTCGAGTTCCAGGAGAA}$ GGGTTTCAAGCCAGAGGAGGCGGTGCAGTTGTGCGAGGCCCTCGAGGCCGCGGGCATGGATTTTGTCGAGACGAGCG 601 GCGGCACCTATGAGAGTTTTGGTTTTGCGCACCGCAAGGAGTCCAGCCGCAAGCGGGAGAACTATTTTATCGAGTTC GCCGAGGTCATCCGCAAGGCCGTCAAGCACATGGTGGTCTACACCACCGGCGGCTTCAAGACGGTGGGCGCCATGGT

 $\tt CGACGCGCTGCAGGGCGTCGATGGGATAGGCATCGGGCGCGCGGAGCCGGAGCCGGACCTCGCCAAGGACATCA$

 ${\tt GCGCAAATAAGGCTGATGGCCAAGGGCGAGGAGCCGTTTGACATCTCAAACGCCGACGAGGTGGCGCGGGTGACGCAGGTGATGGCGGAGGGCAAGGTG}$

- 5 SEQ ID No. 44
 MSPPRFEAAPADPSPLGTPLKYPVSGRSAPNRFLNAAMSEGLATFDEADPSKRGIPTEQLVQLYRRWGQGEWGQIQT
 GNVMIDPEHLEAPGNMVVPRDAEPSGERFDMFSKLAAAAKEHGSLIVAQVGHPGRQARGSVQQHPISASDVQLKQEM
 FGSKFGVPRPATKEDIKAVIEGFAHTAEYLEKAGFDGIELHAAHGYLLAQFLSETTNQRTDEYGGSLENRMRLILEV
 TAEVRRTSKNFILGIKINSVEFQEKGFKPEEAVQLCEALEAAGMDFVETSGGTYESFGFAHRKESSRKRENYFIEF
- 10 AEVIRKAVKHMVVYTTGGFKTVGAMVDALQGVDGIGIGRAAGSEPDLAKDIIAGKVSSIIKYAMGEDEFVLQLTACS AQIRLMAKGEEPFDISNADEVARVTQLMAEGKV

SEQ ID No. 45

- 20 CCCAAGGCCAGTGTCCCGAGCGATCGCCGGATATGCCTGAGTTTCGACGTCATTGCCACGTTTCGA GTGCCCTTCCCCGAATGACTGTCTCCACTATTCGGCAAGATTGTAAATCAAGCCTGAAGAAGCGGAGCATTCTTGGA AGTCGTATGTTCTACTGATTCTGTGCCTGGCGAGACGGGTATATAAAAAGATCACGCACCGAGGAGTTCTTA
- SEQ ID No. 46 25 GTTCGACGTCATTGCCACG

SEQ ID No. 47 CCTTGATCGTTGCTGAGCG

30 SEQ ID No. 48 ATGACTGTCGCCGATATCG

> SEQ ID No. 49 CTATACATCGAAAATAGACTGC

35

SEQ ID No. 50 CCGTCCTGGGCGGAGTATTGGCAGAG

SEQ ID No. 51
40 GCGAATCAGATTCTAGAGGAGCAGGATATCG

SEQ ID No. 52 GCTCAGCACCTCGGCGTCGAAATCTCC

45 SEQ ID No. 53 TCTGCCAATACTCCGCC

SEQ ID No. 54 CTTTCCGGCCGGCATG

50

SEQ ID No. 55
GGTATTGAGGGTCGCATGACTGTCGCCGATATCGA

SEQ ID No. 56 55 AGAGGAGAGTTAGAGCCTACATCGAAAATAGACTGCTTGTACACC

> SEQ ID'No. 57 GGTATTGAGGGTCGCATGTCGCAACCTGTTGTG

60 SEQ ID No. 58
AGAGGAGAGTTAGAGCCTATATCTTCTCGAGTTTCTTCC

SEQ ID No. 59 GGTATTGAGGGTCGCATGGGTTCCAACGCCTTC

SEQ ID No. 60 AGAGGAGAGTTAGAGCCTAAATGGCCCTGCCAAACTG SEQ ID No. 61

GGTATTGAGGGTCGCATGGCTCTCCCTGACGTCGAAA

SEQ ID No. 62 AGAGGAGAGTTAGAGCCTACTCAAAGATGCTCTCC

10 SEQ ID No. 63 GGTATTGAGGGTCGCATGACAGTTCCATACCAAG

SEQ ID No. 64
AGAGGAGAGTTAGAGCCTAATTTACTTCTAATTTAGATGTTC

15 SEQ ID No. 65 GGTATTGAGGGTCGCATGTCGGCAGAAAAGAAG

SEQ ID No. 66
20 AGAGGAGAGTTAGAGCCCAAATCCTTGCACCCTTGCGCC

SEQ ID No. 67 CAGACCAATGGCCAGAAGA

25 SEQ ID No. 68 AGATGGGCGATGTGGTAGTC

5

SEQ ID No. 69 gccgcttacagggaatgata

30
SEQ ID No. 70
atggctcaatctgcgagtct

SEQ ID No. 71 35 CGACTCTTGTGGGTGCTGTA

> SEQ ID No. 72 GTGGAAAACACCCATTCTGG

40 SEQ ID No. 73 CCCCAATCGTCAGATGAAGT

SEQ ID No. 74 CTGGCCCACGATTCACTAAT

45
SEQ ID No. 75
caaaagatcgccatccaact

SEQ ID No. 76
50 Ctggtgacgagtccctcaat

SEQ ID No. 77 ccagcagatgttcgaccccaag

55 SEQ ID No. 78 cagtgaactccatctcgtccatac

SEQ ID No. 79 TCCGTGGCGTCACCTTCC

60
SEQ ID No. 80
CAGATGGGCGATGTGGTAGTC

SEQ ID No 81 tegegegttt eggtgatgae ggtgaaaace tetgacacat geageteeeg gagaeggtea

cagettgtet gtaageggat geegggagea gacaageeeg teagggegeg teagegggtg 120 ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc 180 accatatgeg gtgtgaaata eegcacagat gegtaaggag aaaataeege ateaggegee 240 300 attegecatt caggetgege aactgttggg aagggegate ggtgegggee tettegetat tacgacaget gtetettata cacateteaa ecateatega tgaattttet egggtgttet 3.60 .5 420 cgcatattgg ctcgaattcg agctcggtac ccggggatcc tctagaagtc ctgaatagta 480 .gtttgtggat taacattgtt ccgatgtagg aatcatgatc ccaaccagaa gagctggaca gcccctcttc cagagcattt ttggtgggat gttttggctt agtgcgatgc aactggacaa 540 600 agtecttecg tttetactge gtettacate atetggtate taegeaagee geceaettae 10 660 catatgaata agaggcactc aggttttccc tcacccccc gaagcgatgg taagcgggtg ccaaatgcat cgggagtttc tctatcataa taacctaggt attccgtaat ctattaccag 720 780 tctttccgaa gagctggtag caactgcacg agatttgtag gagcgagtac ccggctggac gagcacgcag cacggctatt ggtcagcatg gtagctaccg aggggaggca ggccgcccaa 840 900 atatcqtqaq tctcctqctt tqcccqqtqt atgaaaccqq aaaagctqct atagagcttc 960 15 tgggcggcgc atgtcgggaa accagcagca agctgaccca gaaagacccg tcctcaagcc attaccgtac taatcaatta tttgtgtagc aacactggga agctgtagtg cataggctgg 1020 1080 agcagetatt tggcctttag eccegtetgt eegeceggtg tgeggttteg actggegege 1140 aagctcaagg tgatcaggtc gttgcgtcag tcggagacaa caagccattg ccttttctac 1200 tgcccctccc ccgctggtgg cctttttctc tcatcttctc ctctcttccc atcatcagca 20 tcattaatct actgtctctc tttctttcta tcattctata aagtaagaac atatccatct 1260 1320 tccctcaatc ccgtctacaa tagtgtcctc ttcactactc tgtctctatc tctcaaagct tgactgacat ttaccccgct cagtaccaga cgaatctaca cagaattcga gctcactaaa 1380 1440 ccatggccaa gttgaccagt gccgttccgg tgctcaccgc gcgcgacgtc gccggagcgg 1500 togagttotg gaccgaccgg ctcgggttot cccgggactt cgtggaggac gacttcgccg gtgtggtccg ggacgacgtg accetgttca tcagcgcggt ccaggaccag gtggtgccgg 25 1560 1620 acaacacct ggcctgggtg tgggtgcgcg gcctggacga gctgtacgcc gagtggtcgg aggtcgtgtc cacgaacttc cgggacgcct ccgggccggc catgaccgag atcggcgagc 1680 agoogtgggg gogggagtto goootgogog accoggoogg caactgogtg cacttogtgg 1740 1800 ccgaggagca ggactgagaa ttccactagt gcagaaagct gttttccttg ctctgtggta 1860 30 taagtctagt gccactattc tatgatgagt tgatgactct ttcatgactg gaaggcttac attotocaag atcatgtoto actoaaaact tatotogggt toactttogg gttocatata 1920 1980 tctcatcatt tctgggttta gaaacatctc tctcgttttt gcagctcttc tacgtactcc 2040 tagcggtttc actgaaatga atacatttgg gtaacctaat tgccaattca tatcttcctg agggcagtaa cacatcacgt acattctatc agctgtgata gagttacaaa actagcaata 2100 35 2160 cttttatgct tectecttte ttaccattta cacateeget ttetetetge tettgatett ggcccctgat tgtattgtca cctcaccaaa ttcaagtcat cacctcttct ctagagtcga 2220 2280 cttttatgga cagcaagcga accggaattg ccagctgggg cgccctctgg taaggttggg 2340 aagccctgca aagtaaactg gatggctttc tcgccgccaa ggatctgatg gcgcagggga 2400 tcaagctctg atcaagagac aggatgagga tcgtttcgca tgattgaaca agatggattg 40 2460 cacgcaggtt ctccggccgc ttgggtggag aggctattcg gctatgactg ggcacaacag 2520 acaatcggct gctctgatgc cgccgtgttc cggctgtcag cgcaggggcg cccggttctt 2580 tttgtcaaga ccgacctgtc cggtgccctg aatgaactgc aagacgaggc agcgcggcta tcgtggctgg ccacgacggg cgttccttgc gcagctgtgc tcgacgttgt cactgaagcg 2640 2700 ggaagggact ggctgctatt gggcgaagtg ccggggcagg atctcctgtc atctcacctt 45 gctcctgccg agaaagtatc catcatggct gatgcaatgc ggcggctgca tacgcttgat 2760 ccggctacct gcccattcga ccaccaagcg aaacatcgca tcgagcgagc acgtactcgg 2820 2880 atggaagccg gtcttgtcga tcaggatgat ctggacgaag agcatcaggg gctcgcca gccgaactgt tcgccaggct caaggcgagc atgcccgacg gcgaggatct cgtcgtgacc 2940 catggcgatg cctgcttgcc gaatatcatg gtggaaaatg gccgcttttc tggattcatc 3000 50 3060 gactgtggcc ggctgggtgt ggcggaccgc tatcaggaca tagcgttggc tacccgtgat 3120 attgctgaag agcttggcgg cgaatgggct gaccgcttcc tcgtgcttta cggtatcgcc gctcccgatt cgcagcgcat cgccttctat cgccttcttg acgagttctt ctgaattatt 3180 aacgcttaca atttcctgat geggtatttt ctcgcatgca tcactagtga attcgcggcc 3240 gectgeaggt egacetgeag geatgeaage ttgecaaega etaegeaeta gecaaeaaga 3300 55 3360 gcttcagggt tgagatgtgt ataagagaca gctgtcttaa tgaatcggcc aacgcgcggg 3420 gagaggcggt ttgcgtattg ggcgctcttc cgcttcctcg ctcactgact cgctgcgctc ggtcgttcgg ctgcggcgag cggtatcagc tcactcaaag gcggtaatac ggttatccac 3480 agaatcaggg gataacgcag gaaagaacat gtgagcaaaa ggccagcaaa aggccaggaa 3540 3600 ccgtaaaaag qccgcgttqc tggcgttttt ccataggctc cgccccctg acgagcatca 3660 60 caaaaatcga cgctcaagtc agaggtggcg aaacccgaca ggactataaa gataccaggc gtttccccct ggaagctccc tcgtgcgctc tcctgttccg accctgccgc ttaccggata 3720 cctgtccgcc tttctccctt cgggaagcgt ggcgctttct catagctcac gctgtaggta 3780 3840 tctcagttcg gtgtaggtcg ttcgctccaa gctgggctgt gtgcacgaac cccccgttca 3900 gcccgaccgc tgcgccttat ccggtaacta tcgtcttgag tccaacccgg taagacacga 65 cttatcgcca ctggcagcag ccactggtaa caggattagc agagcgaggt atgtaggcgg 3960

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	caaacaaacc	accgctggta	gcggtggttt	ttttgtttgc	aagcagcaga	ttacgcgcag	4140
_	aaaaaaagga	tctcaagaag	atcctttgat	cttttctacg	gggtctgacg	ctcagtggaa	4200
5	cgaaaactca	cgttaaggga	ttttggtcat	gagattatca	aaaaggatct	tcacctagat	4260
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	agtgctcatc	attggaaaac	gttcttcggg	gcgaaaactc	tcaaggatct	taccgctgtt	5040
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	ggcgacacgg	aaatgttgaa	tactcatact	cttccttttt	caatattatt	gaagcattta	5220
	tcagggttat	tgtctcatga	gcggatacat	atttgaatgt	atttagaaaa	ataaacaaat	5280
	aggggttccg	cgcacatttc	cccgaaaagt	gccacctgac	gtctaagaaa	ccattattat	5340
	catgacatta	acctataaaa	ataggcgtat	cacgaggccc	tttcgtc		5387
25					-		
	SEO TO NO	82					

SEQ ID No. 82

CTCCCAAGCTTGTCTTA

ATGACAGTTCAATCACAGCAACAATCCCAGGCTATTCCCGTCCTTTCTTCCCAGAATGGCACTGAACCCCAAGACGC $\verb|AAACAAGGAGGTTGTTCAGAATGTCGCTGCCAAAGGAGTGCAATACTTCAACCCTGAGCAACTTCCTGCACCAGGTC| \\$ TCGGTATAAACGGTCCCAATAATACTCTACCAAAGGTCTTTACACCCATCAAGATTCGCGGCATGACCATGCCCAAC 30 $\tt CGTATCTGGGTCAGCCCCATGTGCCAATACAGTGCCCGTGACGGCTTTCAGCAGCCTTGGCACTTTGCCCACTACGG$ $\tt CGGACTGGCCCAACGTGGCCCTGGCCTCATCATGCTAGAAGCTACCGCAGTTCAAGCACGTGGCCGTATCACACCTG$ AAGATTCTGGCATCTGGCTAGACTCTCATGTTGAGGGACTGCGAAAGCACGTCGAGTTTGCCCATGCCAACAACTCT $\tt CTTATCGGTATCCAGATTGGCCATGCTGGTCGCAAGGCCTCCTGCGTTGCTCCTTGGTTAGACGCCGGACTTGCCGC$ TGAAAAGGCCGCTGGTGGATGGCCCGATGACGTTGTCGGACCTAGCAACGAGCCTTTTGCTCCTGGCTACCC 35 CCCGTGCTATTACTCTTGAAGAGATTGAACAGTTGAAGGAGGACTTTGTTTCCGGTGTTCGTCGAGCGGTTGAAGCA GGATTTGACACTATCGACTTCCATTTCGCTCACGGTTATCTTGTTTCCAGCTTCCTGTCCCCTGCCACCAACAAGCG TACCGACAAGTACGGAGGTAGCTTCGAGAACAGAGTGCGCCTTGCTCTCGAGATTGTCGAGGCTGCACGAGCTGTTA ACCTGGACTCTTGAGCAGAGCATCAAGCTTGCACACCAGTTAGCAGACCGTGGTGTCGATGTTTTGGATGTTTCCAG 40 TGGTGGCATCCACAAGATGCAAAAGGTCGCTGCTGGTCCCGGTTACCAGGCACCTCTTGCCAAGGCGATCAAGAAGT CAGTTGGAGACAAGATGTTGATCAGCACTGTTGGTAGCATCAAGATAGGTACCCTTGCGGAGGAGATCATCGCTGGA ${\tt GGAGAGGACGATACCCCCTTGGATCTTGTGGCTTCAGGCCGTCTGTTCCAGAAGAACACTGGACTTGTTTGGTCATG}$ GGCTGACGATCTGAACACTTCTATCCAGATCGCTCATCAGATCGCATGGGGTTTCGGTGGCAGAGCTAAGAAGAACG

45

SEQ ID No. 83
FG00074.1 hypothetical protein 3813139459+
MTVQSQOOSOAIPVLSSONGTEPODANKEVVONVAAKGVOYFN

MTVQSQQQSQAIPVLSSQNGTEPQDANKEVVQNVAAKGVQYFNPEQLPAPGLGINGPNNTLPKVFTPIKIRGMTMPN
RIWVSPMCQYSARDGFQQPWHFAHYGGLAQRGPGLIMLEATAVQARGRITPEDSGIWLDSHVEGLRKHVEFAHANNS
LIGIQIGHAGRKASCVAPWLDAGLAAEKAAGGWPDDVVGPSNEPFAPGYPTPRAITLEEIEQLKEDFVSGVRRAVEA
GFDTIDFHFAHGYLVSSFLSPATNKRTDKYGGSFENRVRLALEIVEAARAVMPEDMPLFTRISGTDWLENNPEYEGE
TWTLEQSIKLAHQLADRGVDVLDVSSGGIHKMQKVAAGPGYQAPLAKAIKKSVGDKMLISTVGSIKIGTLAEEIIAG
GEDDTPLDLVASGRLFQKNTGLVWSWADDLNTSIQIAHQIAWGFGGRAKKNAPKLVL

10 SEQ ID No. 85

MDTSRFVSGLTPPLVDSIDALKISNFVPTRSGHPPPGSVPESILPEGVKKPALFQTLTLPFAAPEQAGKMTFKNRII
VSPMCQYSANNGLPTPYHIAHLGSFALHGVGNVMVEASGVEPEGRITPQDLGIWSEQHRDAHKALVSVLKSFTDGLG
VGLQLAHAGRKASDWSPFYRGEKKQKFVTQEEGGWPDRVVAPSAIAYAQGHVTPRALTTEDINKLQDKFVQSARWAF
EAGYDYVELHSAHGYLMHSFLSPLTNQRTDEYGGSLENRARFLLNVARRIRQEFPNKGLWVRVSSTDWADQAHQADS
WTVDQTVELAKMLQEARVDLLDVSSGGLVPFQKITVGAGYQLFGAKAVRDALAKIEPDASKRMLVGAVGMMEGSYDS
PNGQDRSQIGKLAEQSIQSGECDAVLLARGLMSYPSWTEDASVALMGTRAAGNPQYHRVHVAKK

SEQ ID No. 86

5

- MSALFEPYTLKDVTLRNRIAIPPMCQYMAEDGLINDWHQVHYASMARGGAGLLVVEATAVAPEGRITPGCAGIWSDA
 20 HAQAFVPVVQAIKAAGSVPGIQIAHAGRKASANRPWEGDDHIGADDARGWETIAPSAIAFGAHLPNVPRAMTLDDIA
 RVKQDFVDAARRARDAGFEWIELHFAHGYLGQSFFSEHSNKRTDAYGGSFDNRSRFLLETLAAVREVWPENLPLTAR
 FGVLEYDGRDEQTLEESIELARRFKAGGLDLLSVSVGFTIPETNIPWGPAFMGPIAERVRREAKLPVTSAWGFGTPQ
 LAEAALQANQLDLVSVGRAHLADPHWAYFAAKELGVEKASWTLPAPYAHWLERYR
- SEQ ID No. 87.

 MSALFEPFRLRDTTIPNRIWMPPMCQYSAAPEGPSAGVPGDWHFAHYGARAVGGTGLIVVEATGVSPEGRISPQDLG
 LWNDTQVEAFRRITGFLRSQGTVPAVQLAHAGRKASTAQPWRGGAPVGADAYGWQPLAPSALAFDERHPVPTELTVP
 QIQEAVGRFADAARRALAAGFEIAEIHGAHGYLIHEFLSPHSNQRTDAYGGSYANRTRFALEVVDAVREVWPDDKPL
 FFRVSATDWLEEGGWTPDDTVRFARDLEAHGIDLLDVSTGGNVPRVRIPTGPGYQVPFAARVKAGSTLPVAAVGLIT
 30 EPGQAEKILANGEADAVLLGRELLRNPSWAQHAARELGVDARMPDQYGWGM
- SEQ ID No. 88

 MTVSSAAAPQPASPAAPLLFTPLKLRSLELPNRVVVSPMCTYSATDGVANEFHLVHLGQYALGGAGLILAEATAVSP
 EGRITPEDLGLWDDRQIVPLGHITDFVHQHGGHIGVQLAHAGRKASTYAPWRGKGAVPAELGGWQVIGPDENSFHDL

 55

 FPTPAMMGADELRGVVDAFSAAARRAQVAGFDAVEVHAAHGYLLHQFLSPLANTRTDDYGGSFENRTRLLLEVVRÅV
 RHVWPAHLPLFVRLSATDWAEGGWDLEQTVQLSKLLKYEGVDVLDISSGGLTAAQQIEVGPGYQVPFAAAVSRAETE
 ISVMAVGLIETGAQAEAILQAGDADLIALGRPFLRDPHWAQRAARELGLRPVSIDQYARAGW

SEQ ID No. 89

- 40 MRIVCIGGGPAGLYFAILMKKLNPAHEIRVIERNRPYDTFGWGVVFSDATMDNMREWDSETADAIQVAFNHWDDIEL HFKGRTIRSGGHGFVGIGRKMMLNILQARCEELGVELVFDREVESDAEFPDADLVIASDGINSRIRNKYAEVFKPDI VTRPNRYIWLGTTKLFDAFTFFFEKTEHGWFQAHIYKFDDKTTTFIVECPEHVWKAHGLDTADQEQSIAFCEQLFGK HLDGHRLMTNSRHLRGSAWLNFQRVKCEQWHHYNGKSHVVLMGDAVHTAHFAIGSGTKLALEDAIELTRLFRDEGDT REHIPAVLERYQAARNIDVLRLQNAAWNAMEWFEVCGARYCDTLEPEQFMYSMLTRSQRISHENLRLRDAGWLEGYE
- 45 RWLARKAGMTVRDDETPPPMFTPFKLRGLTLANRIVMSPMAMYSAEDGAPTDFHLVHFGSRALGGAGLLYTEMTCV SPDARITPGCAGMYKPEHVNAWKRIVDFVHGNSDAKIGMQLGHAGRKGATKLAWEGIDEPLEAGAWELISASPLPYL PHSQVPRAMTRDDMERVRNDFVRATRMAAEAGFDILELHCAHGYLLSSFLSPLTNRRTDEFGGDLENRARFPLEVFK AMRAMWPTNRPMSVRLSCHDWFPGGNTADDAVAIARLFKEAGADIIDCSSGQVWKGDQPVYGRMYQTPFADRIRNEV GIPTLAVGAISEADHANSIIAAGRADLCAIARPHLADPAWTLHEAAKIGFGEVAWPKQYRSARGQYETNLQRAAAAV AGK

- SEQ ID No. 90

 MREEPSSAQLFKPLKVGRCHLQHRMIMAPTTRFRADGQGVPLPFVQEYYGQRASVPGTLLITEATDITPKAMGYKHV
 PGIWSEPQREAWREIVSRVHSKKCFIFCQLWATGRAADPDVLADMKDLISSSAVPVEEKGPLPRALTEDEIQQCIAD

 FAQAARNAINAGFDGVEIHGANGYLIDQFTQKSCNHRQDRWGGSIENRARFAVEVTRAVIEAVGADRVGVKLSPYSQ
 YLGMGTMDELVPQFEYLIAQMRRLDVAYLHLANSRWLDEEKPHPDPNHEVFVRVWGQSSPILLAGGYDAASAEKVTE
 QMAAATYTNVAIAFGRYFISTPDLPFRVMAGIQLQKYDRASFYSTLSREGYLDYPFSAEYMALHNFPV
- SEQ ID No. 91

 MTIRKLDGEESMLFQPLEIANGRIRLSHRVVHAPMTRNRGVPLNPTSTPEQPNRIWYPGDLMVQYYRQRATPGGLII
 SEGVPPSLESNGMPGVPGLWTPEQAAGWKRVVDAVHEQGGYIYCQLWHAGRATIPQMTGSPAVSASATVWDSPTECY
 SHPPVGSTEPVRYADHPPIELTIPHLKQTIRDYCNAAKTAMEIGFDGVELHAGNGYLPEQFLSSNVNKRTDEYGGSP
 EKRCRFVLELMDELAATVGEDNLAIRLSPFGLFNQARGEQRVETWTFLCESLKKAHPNLSYVSFIEPRYEQIFSYEE
 KDNFLRSWGLSDVDLSSFRKIFGTTPFFSAGGWDQSNSWGVLEEGRYDALLYGRYFTSNPDLVERLRKGIPFTPYDR

65 SRFYGPFEDNAKCYVDYPPATASS

SEQ ID No. 92

MTVESTNSFVVPAGTKQIEIAPLGSTKLFQPIKVGKNILPHRVAHAPTTRFRAAKNHTPSDLQLEYYKTHSQYPGTL
IITEATFTSEQGGMDLHVPGIYNDAQTKAWKKINDEIHANGSFSSMQLWYLGRVANPKDLKDAGLPLIGPSAVYWDE
ESEKLAKSVGNELRELTEKEIDHIVEVEYPNAAKRAIEAGFDYIEVHSAPGYFLDQFLNPASNKRTDKYGGSIENRA
RLLLRIIDKLIGIVGAEKLAVRLAPWSSFLGMEIEGEEIHSYILQQLQQRADNGQQLAYVSLIEPRVIGIFDASLED
QKGRSNEFAYKYWKGNFVRAGNYTYDAPEFKTLLHDLDNDRTIVGFARFFTSNPDLVEKLKLGKPLNHYDREEFYKY
YNYGYNSYDESEKQVIGKPLV

10 SEQ ID No.

MTIESTNSFVVPSDTKLIDVTPLGSTKLFQPIKVGNNVLPQRIAYVPTTRFRASKDHIPSDLQLNYYNARSQYPGTL IITEATFASERGGIDLHVPGIYNDAQAKSWKKINEAIHGNGSFSSVQLWYLGRVANAKDLKDSGLPLIAPSAVYWDE NSEKLAKEAGNELRALTEEEIDHIVEVEYPNAAKHALEAGFDYVEIHGAHGYLLDQFLNLASNKRTDKYGCGSIENR ARLLLRVVDKLIEVVGANRLALRLSPWASFQGMEIEGEEIHSYILQQLQQRADNGQQLAYISLVEPRVTGIYDVSLK

15 DQQGRSNEFAYKIWKGNFIRAGNYTYDAPEFKTLINDLKNDRSIIGFSRFFTSNPDLVEKLKLGKPLNYYNREEFYK
YYNYGYNSYDESEKQVIGKPL

SEQ ID No. 94

- MAATAAESRLFQPLKLTPKITLGHRLAMAPLTRFRSDDEHVPIVPLMTTYYSQRASVPGTLLVTEATFISPAAGGYD
 20 NVPGIYNAAQIAAWKKITDAVHAKGSFIFCQLWSLGRAANPEVLAKEGGLKLKSSSAVPMEEGAPVPEEMTVAEIKE
 RVAEYAAAAKNAVEAGFDGVEIHGANGYLIDQFLQDTCNQRTDEYGGSIENRSRFAHEVVKAVVEAVGAEKTGIRLS
 PYSTFQGMKMKKDLIPQFEDVIRKINGFGLAYLHLTQSRVAGNMDVQPEEDEENLAFAAKLWDGPLLIAGGLTPETA
 KHLVDREFPEKDVVATFGRHFISTPDLPFRIKEGIELNPYDRDTFYKAKSPDGYIDQPFSKEFEKVYGAOA
- 25 SEQ ID No. 95

 MSFVKDFKPQALGDTNLFKPIKIGNNELLHRAVIPPLTRMRALHPGNIPNRDWAVEYYTQRAQRPGTMIITEGAFIS
 PQAGGYDNAPGVWSEEQMVEWTKIFNAIHEKKSFVWVQLWVLGWAAFPDNLARDGLRYDSASDNVFMDAEQEAKAKK
 ANNPQHSLTKDEIKQYIKEYVQAAKNSIAAGADGVEIHSANGYLLNQFLDPHSNTRTDEYGGSIENRARFTLEVVDA
 LVEAIGHEKVGLRLSPYGVFNSMSGGAETGIVAQYAYVAGELEKRAKAGKRLAFVHLVEPRVTNPFLTEGEGEYEGG
 30 SNDFVYSIWKGPVIRAGNFALHPEVVREEVKDKRTLIGYGRFFISNPDLVDRLEKGLPLNKYDRDTFYQMSAHGYID
 YPTYEEALKLGWDKK

SEQ ID No. 96

MPFVKDFKPQALGDTNLFKPIKIGNNELLHRAVIPPLTRMRAQHPGNIPNRDWAVEYYAQRAQRPGTLIITEGTFPS

PQSGGYDNAPGIWSEEQIKEWTKIFKAIHENKSFAWVQLWVLGWAAFPDTLARDGLRYDSASDNVYMNAEQEEKAKK
ANNPQHSITKDEIKQYVKEYVQAAKNSIAAGADGVEIHSANGYLLNQFLDPHSNNRTDEYGGSIENRARFTLEVVDA
VVDAIGPEKVGLRLSPYGVFNSMSGGAETGIVAQYAYVLGELERRAKAGKRLAFVHLVEPRVTNPFLTEGEGEYNGG
SNKFAYSIWKGPIIRAGNFALHPEVVREEVKDPRTLIGYGRFFISNPDLVDRLEKGLPLNKYDRDTFYKMSAEGYID
YPTYEEALKLGWDKN

4 Ó

SEQ ID No. 97.

MPFVKGFEPISLRDTNLFEPIKIGNTQLAHRAVMPPLTRMRATHPGNIPNKEWAAVYYGQRAQRPGTMIITEGTFIS PQAGGYDNAPGIWSDEQVAEWKNIFLAIHDCQSFAWVQLWSLGWASFPDVLARDGLRYDCASDRVYMNATLQEKAKD ANNLEHSLTKDDIKQYIKDYIHAAKNSIAAGADGVEIHSANGYLLNQFLDPHSNKRTDEYGGTIENRARFTLEVVDA

- 45 LIETIGPERVGLRLSPYGTFNSMSGGAEPGIIAQYSYVLGELEKRAKAGKRLAFVHLVEPRVTDPSLVEGEGEYSEG
 TNDFAYSIWKGPIIRAGNYALHPEVVREQVKDPRTLIGYGRFFISNPDLVYRLEEGLPLNKYDRSTFYTMSAEGYTD
 YPTYEEAVDLGWNKN
- SEQ ID No. 98 50 GCTAGCATGACTGTCGCCGATATCGA

SEQ ID No. 99 GCTAGCCTATACATCGAAAATAGACTGC

55 SEQ ID No. 100 ACTAGTCCAGGGGACTGTCGTGGTCAA

SEQ ID No. 101 CAATTGCCCAGGCCTAATGCATGCTG

CLAIMS

- 1. Method of identifying an anti-fungal agent which targets an essential protein or gene of a fungus comprising contacting a candidate substance with
- (i) a NADH:flavin oxidoreductase protein which comprises the sequence shown by SEQ ID NO:3,
- (ii) a NADH:flavin oxidoreductase protein which is a homologue of (i) and which comprises the sequence shown by SEQ ID NO: 8, 12,-14, 19, 24, 42, 44, 83 or 85,
 - (iii) a protein which has 50% identity with (i) or (ii),

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- (iv) a protein comprising a fragment of (i), (ii) or (iii) which fragment has a length of at least 50 amino acids,
 - (v) a polynucleotide that comprises sequence which encodes (i), (ii), (iii) or (iv),
- (vi) a polynucleotide comprising sequence which has at least 70% identity with the coding sequence of (v), and determining whether the candidate substance binds or modulates (i), (ii), (iii), (iv), (v) or (vi), wherein binding or modulation of (i), (ii), (iii), (iv), (v) or (vi) indicates that the candidate substance is an anti-fungal agent.
- 2. Method according to claim 1 wherein (iii) or (iv) have an oxidoreductase activity.
 - 3. Method according to claim 1 or 2 wherein (i), (ii), (iii) or (iv) comprise one or more of the motifs defined by regions 1 to 11 in Figures 1 and 2.
- 4. Method according to any one of the preceding claims comprising carrying out a redox reaction in the presence and absence of the candidate substance to determine whether the candidate substance inhibits the oxidoreductase activity of a protein as defined in any one of the preceding claims, wherein the redox reaction is carried out by contacting said protein with NADH or NADPH; and an electron acceptor, under conditions in which in the absence of the candidate substance the protein catalyses reduction of the electron acceptor.

- 5. Method according to any one of the preceding claims wherein (iii) is a protein comprising the sequence of any of the following: SEQ ID NO: 6, 10, 16, 22, 27, 30, 33, 35, 38, 40.
- 6. Method according to any one of the preceding claims wherein the (i) or (ii) is an oxidoreductase of Aspergillus flavus; Aspergillus fumigatus; Aspergillus nidulans; Aspergillus niger; Aspergillus parasiticus; Aspergillus terreus; Blumeria graminis; Candida albicans; Candida cruzei; Candida glabrata; Candida parapsilosis; Candida tropicalis; Colletotrichium trifolii; Cryptococcus neoformans; Encephalitozoon cuniculi; Fusarium graminarium; Fusarium solani; Fusarium sporotrichoides; Leptosphaeria nodorum; Magnaporthe grisea; Mycosphaerella graminicola; Neurospora crassa; Phytophthora capsici; Phytophthora infestans; Plasmopara viticola; Pneumocystis jiroveci; Puccinia coronata; Puccinia graminis; Pyricularia oryzae; Pythium ultimum; Rhizoctonia solani; Schizzosaccharomyces pombe;
 Trichophyton interdigitale; Trichophyton rubrum; or Ustilago maydis.
 - 7. Method according to any one of the preceding claims which further comprises formulating the identified anti-fungal agent into a agricultural or pharmaceutical composition.
 - 8. Method according to any one of claims 1 to 6 which further comprises killing or impairing the growth of a fungus by contacting the fungus with the identified antifungal agent.
- 9. Use of (i), (ii), (iii), (iv), (v) or (vi) as defined in any one of claims 1 to 6 to identify or obtain an anti-fungal agent.
 - 10. Use of an anti-fungal agent identified by the method of any one of claims 1 to 6 in the manufacture of a medicament for prevention or treatment of fungal infection.

- 11. Method of detecting the presence of a fungus in a sample comprising detecting the presence in the said sample of a protein or polynucleotide as defined in any one of claims 1 to 3, 5 or 6.
- 5 12. Method according to claim 11 wherein the sample is from an human, animal or plant individual who is suspected of having a fungal infection.
 - 13. An isolated protein or polynucleotide as defined in any one of claims 1 to 3, 5 or 6.

- 14. A vector comprising a polynucleotide as defined in any one of claims 1 to 3, 5-or 6.
- 15. A recombinant cell comprising a polynucleotide as defined in any one of claims 1 to 3, 5 or 6 or a vector according to claim 14.
 - 16. A method of obtaining a protein as defined in any one of claims 1 to 3, 5 or 6 comprising expressing the protein from a polynucleotide as defined in any one of claims 1 to 3, 5 or 6 or a vector according to claim 14.

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- 17. A method of obtaining a polynucleotide as defined in claim 1 to 3, 5 or 6 comprising replication of a vector as defined in claim 14 or synthesis of the polynucleotide by condensation of nucleotides.
- 25 18. An organism which is transgenic for a polynucleotide as defined in any one of claims 1 to 3, 5 or 6.
 - 19. An organism which has been genetically engineered to render a polynucleotide or protein as defined in any one of claims 1 to 3, 5 or 6 non-functional or inhibited.

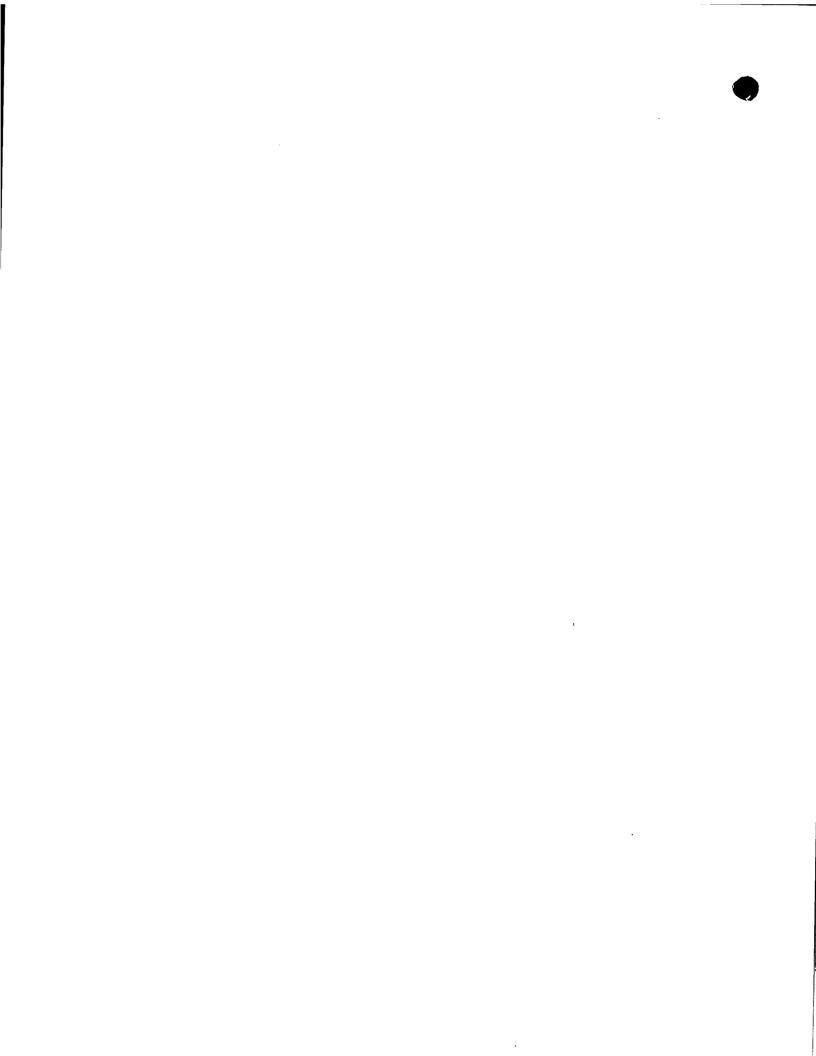
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20. An antibody which is specific for a protein as defined in any one of claims 1 to 3, 5 or 6.

- 21. A method for preventing or treating a fungal infection comprising administering an anti-fungal agent identified by the method of any one of claims 1 to 6.
- 5 22. A method for preventing or treating a fungal infection comprising administering a protein or polynucleotide as defined in any one of claims 1 to 3, 5 or 6.
 - 23. A method of killing, or impairing the growth of, a fungus comprising inhibiting the expression or activity of a polynucleotide or protein as defined in any one of claims 1 to 3, 5 or 6.

- 24. A method according to claim 23 wherein the fungus has infected a human, animal or plant individual.
- 25. A fungus which has been killed, or whose growth has been impaired, by inhibition of the expression or activity of a protein or polynucleotide as defined in any one of claims 1 to 3, 5 or 6.

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			MM(A)D	TOUPPREGIP	YETPRONPPA	GTAANPOTN-	GOKIPKLE	TPLTIR-GVT	FQ	NRLGLAPLCQ
SEQ 3 SEQ 6										
SEQ 8										
SEQ 10										
SEQ 12			MALDAUKEDE	DETKGAPEVS	YYTPEOPVPA	GTFYPOSSD-	EVAPATE	OLDER CONTRACTOR	TL	MICTOADTING
SEQ 14										
SEQ 16			MADETQKK	TSSPAAPGVP	FYTPAQVPAA	GTPLPSTPG-	DVETLE	TPLKIR-GVE QPLTLPNGLT	LP	NRIVKAAMAE
SEQ 19			*************	my cumpt CVP	AAMDDOLDDD	CTRIOCODA-	TPTLF	KPLKIR-GVE	LS	NREGVSPMCT
SEQ 22										
SEQ 24 SEQ 27			MTG	TANKAAPGVP	FYTPAOEPPA	GTPVDASTA-		KPLKIK-DLI	TMenner	MUTHABELION
SEQ 30			MAYEI	IDNVAALGVP	YYTPAQDPPA	Grorag	BIKDE	TETTTIC GAY		
SEQ 33										
SEQ 35		МР	KCEANGHHKI	IINKEAPNVP	FYTPVQDPPA	GTSYDVQPEG	SLF	SLIKIR-NLT	TO	NRIFVSPMCQ
SEQ 38			TORK	TONTABEGAP	YYTPAOD, PA	GTOTSGST	KVF	T.ITIK-GVT	E P	MUDE THE DOO
SEQ 40 '								LKIR-GLT	rd	NRIMERGECO
SEQ 42						DDDDCDT 5	mprvv	DVSCDSVD		NRFLNAAMSE
SEQ 44					-MSPPRELAA	CT CTNCDNNT	TPKVF	PVSGRSAP TPIKIR-GMT	мр	NRIWVSPMCO
SEQ 83	MTVQSQQQSQ	AIPVLSSQNG	TEPODANKEV	VORTONTATE	TEMBERGREVE	DDGGVPESTI.	PEGVKKPALE	QTLTLP-FAA	PEOAGKMTEK	NRIIVSPMCQ
SEQ 85		PDTS	REVSGETPPE	ADSTDAUKTS	MEVELKSONE					
Bacteria T44612							MSALF	EPYTLK-DVT	LR	NRIAIPPMCQ
NP 625402							MSALF	EPFRLR-DTT	IP	NKTAWESWCO
NP 295913						ADODASDAD -	PLLF	TPLKER-SEE	LP	NRVVVSPMCT
AF320254			DECETEURNT.	DT.DDDGGJT.EG	YERWI. ARKAG	MTVRDDETP-	PPPMF	TPFKLR-GLT	LA	NKIVMSPMAM
OYE family										
A£4875						MREEPSSAQ-	LF	KPLKVGRC	HLQ	HRMIMAPTTR
Af4961					MTT	RKLDGEESM⇔		OBPETW-URK	TKT2	HELY A LIMIT LAYER
Ca2460				MTVESTNS	evvpagtkqi	EIAPLGSTK-		QPIKVG-KNI	I.G.	HRIAMAPIAR
Nc4452						MAATAAESH-		QPLKLTPKIT KPIKIG-NNE	I.I	HRAVIPPLTR
ScOYE1				MS	EAKDE	KDONT COLN-		KPIKIG-NNE	LL	HRAVIPPLTR
SCOYE2				MP	EVKCF	EPISIRDIN-	LF	EPIKIG-NTQ	LA	HRAVMPPLTR
SCOYES				MTTESTNS	FVVPSDTKTT	DVTPLGSTK-	LF	ÖЪIKΛ Θ−ΝИΛ	LP	QRIAYVPTTR
A36990										
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SEQ 6 SEQ 8	2 ** YSA YSA YSCES	QDGHM DDGHM DPSSPHVGAL	121 3 TDYHIAHL TPWHMAHL TNYHIAHL	*GGIAQRGPGL GGIAQRGPGF GHLALKGAGL	MLIEATAVQP IMVEATAVEP VFIEATAVQP	E-GRITPODU N-GRISPNDS	GLWK-DS GLWK-DS GLWQ-DG	QIAPMRQIEPLS TTSEQFLGLK	RVI-DFVHSQ RVI-EFVHSQ RVV-EFMHAQ RVI-DFVHSO	-5 **** GQ-KIGVQ NQ-LIGVQ GA-KVGIQ SO-KIGVQ
SEQ 6 SEQ 8 SEQ 10	2 ** YSA YSCES YSA	QDGHMDDGHM DPSSPHVGAL	121 3 TDYHIAHL TPWHMAHL TNYHLAHL TDYHIAHL	* GGIAQRGPGL GGIAQRGPGF GHLALKGAGL GGIAQRGPGL	MLIEATAVQP IMVEATAVEP VFIEATAVQP MMIEATAVSP	E-GRITPQDV E-GRITPQDV N-GRISPNDS E-GRITPQDV	GLWKDSGLWQDGGLWKDS	QIAPMRQIEPLS TTSEQFLGLKQIAPMK	RVI-DFVHSQ RVI-EFVHSQ RVV-EFMHAQ RVI-DFVHSQ PTV-DYAHSO	-5
SEQ 6 SEQ 8 SEQ 10 SEQ 12	2 ** YSA YSCES YSA YSA	QDGHMDDGHM DPSSPHVGALEDGHM	121 TD-YHIAHL TN-YHIAHL TN-YHIAHL TD-YHIAHL TP-YHIAHL	GGIAQRGPGL GGIAQRGPGF GHLALKGAGL GGIAQRGPGL GSLVNRGPGI	MLIEATAVQP IMVEATAVEP VFIEATAVQP MMIEATSVSP TIVESTAVSP	E-GRITPQDV E-GRITPQDL N-GRISPNDS E-GRITPQDV E-GGLSPNDL	GLWKDS GLWQDG GLWKDS GLWKDS	QIAPMRQIEPLS TTSEQFLGLKQIAPMKQAEKLK	RVI-DFVHSQ RVI-EFVHSQ RVV-EFMHAQ RVI-DFVHSQ PIV-DYAHSQ FIV-DFIHDO	-5
SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14	2 **	DPSSPHVGAL DPSSPHVGAL DPSSPHVGAL DYNFEA DYNFEA	121 TD-YHIAHL TN-YHIAHL TN-YHIAHL TD-YHIAHL TP-YHIHY TI-FHFVHY	GGIAQRGPGL GGIAQRGPGF GHLALKGAGL GGIAQRGPGL GSIVNRGPGI GSFAVRGPAL	MLIEATAVQP IMVEATAVEP VFIEATAVQP MMIEATSVSP TIVESTAVSP IILESIFVSE TIFEBTGVLP	E-GRITPQDV E-GRITPQDV N-GRISPNDS E-GRITPQDV E-GGLSPHDL N-SGLSIHDL N-GRITPECS	GLWK-DSGLWC-DGGLWK-DSGLWK-DEGLWN-DDGLWO-DS	QIAPMRQIEPLS TTSEQFLGLKQAEKLKQAEKLKQAHSLR	RVI-DFVHSQ RVI-EFVHSQ RVV-EFMHAQ RVI-DFVHSQ PIV-DYAHSQ KIV-DFIHDQ RIV-DYIHSQ	-5
SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16	2 **	QDGHM DPSSPHVGAL	1213 TDYHIAHL TNYHIAHL TNYHIAHL TDYHIAHL TPYHIHY TLFHFVHY TDWHIVHL	GGIAQRGPGL GGIAQRGPGF GHLALKGAGL GGIAQRGPGL GSIVNRGPGI GSFAVRGPAL GSFALRGVPL YDTWARGDWG	MLIEATAVQP IMVEATAVEP VFIEATAVQP MMIEATSVSP TIVESTAVSP IILESIFVSE TIFEATGVLP LILEGROVOVD	4****** E-GRITPQDV E-GRITPQDV N-GRISPNDS E-GRITPQDV E-GGLSPHDL N-SGLSIHDL N-GRITPECS HAHKGDAHDI	GLWK-DSGLWK-DSGLWC-DGGLWC-DSGLWC-DBGLWC-DBGLWC-DS	QIAPMRQIEPLS TTSEQFLGLK	RVI-DFVHSQ RVI-EFVHSQ RVV-EFMHAQ RVI-DFVHSQ PIV-DYJHSQ KIV-DFIHDQ KIV-DFIHSQ KAWADAARLN	-5
SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14	2		121 TDYHIAHL TDYHIAHL TDYHIAHL TDYHIAHL TDYHIHH TDYHIHH TDWHIHH TLFHFVHY TDWHIVHL PNPELANV	GGIAQRGPGL GGIAQRGPGF GHLALKGAGL GGIAQRGPGL GSIAVRGPGI GSFAVRGPAL GSFALRGVPL YATWARGDWG	MLIEATAVQP IMVEATAVEP VFIEATAVQP MMIEATSVSP TIVESTAVSP IILESIFVSE TIFEATGVLP LILITGNVQVD	4 E-GRITPQDV E-GRITPQDV N-GRISPNDS E-GRITPQDV N-GRISPNDL N-GRISPNDL N-GRISPNDL N-GRITPECS HAHKGDAHDI N-GRISPEDS		QIAPMRQIEPLS TTSEQFLGLK	RVI-DFVHSQ RVI-EFVHSQ RVV-EFMHAQ PVI-DFVHSQ PIV-DYAHSQ KIV-DYIHSQ KIV-DYIHSQ KAWADAARIN RIV-DYVHSQ	-5
SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19	2	QDGHMDDGHM DPSSPHVGAL	121 TDYHIAHL TPWHIAHL TDYHIAHL TDYHIAHL TPYHIHY TLFHFVHY TDWHLVHL ENPELAAV TDFHLVHL	*GGIAQRGPGL GGIAQRGPGF GHLALKGAGL GGIAQRGPGL GSIAVRGPGI GSFAVRGPAL GSFALRGVPL YATWARGDWG GGPALHGTAL CANKERGGGI	MLIEATAVQP IMVEATAVEP VFIEATAVQP MMIEATSVSP TIVESTAVSP IILESIFVSE TIFEATGVLP LILITGNVQVD TIVEATSVTP MMVEATSVTSP	4***** E-GRITPQDV E-GRITPQDV N-GRISPNDS E-GRISPNDV N-SGLSIHDL N-GRITPECS HAHKGDAHDI N-GRISPDNS		QIAPMRQIEPLS TTSEQFLGLK	RVI-DFVHSQ RVI-EFVHSQ RVV-EFMHAQ RVI-DFVHSQ FIV-DYAHSQ KIV-DFIHDQ RIV-DYIHSQ KAWADAARLIN RIV-DYVHSQ RIV-EFAHSQ	-5
SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19 SEQ 22 SEQ 24 SEQ 27	2		121 TD-YHIAHL TP-WHMAHL TP-YHIAHL TP-YHIAHL TP-YHIAHL TP-YHIHH TP-HEVHY TL-FHEVHY TD-WHIVHL PN-PELAAV TD-FHIVHL TD-FHIVHL TD-FHIVHL	GGIAQRGPGL GGIAQRGPGF GHLALKGAGL GGIAQRGPGL GSIVNRGPGI GSFAVRGPAL GSFALRGVPL YATWARGDWG GGPALHGTAL GAMGMRGPGL	MLIEATAVQP LIMEATAVQP VFIEATAVQP MTIEATSVSP TIVESTAVSP LILESIFVSE LILEGIFVSE LILIGNVQVD TIVEATSVTP VMVEATAVSP	E-GRITPQDV E-GRITPQDV N-GRISPNDS E-GRITPQDV E-GGLSPHDL N-SGLSIHDL N-GRITPECS HAHKGDAHDI N-GRISPEDS E-GRISPNDS			RVI-DFVHSQ RVI-EFVHSQ RVV-EFMHAQ RVI-DFVHSQ PIV-DYAHSQ KIV-DFIHDQ RIV-DYIHSQ KAWADAARIN RIV-DYVHSQ RIV-EFAHSQ RIV-EFAHSQ	-5
SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEC 19 SEC 22 SEQ 24 SEQ 27 SEQ 27 SEQ 30	2		1213 TDYHARL TPWHMAHL TPYHLAHL TPYHLHIY TPYHLHIY TDHEVIVHL ENPELDAV TDFHLWHL TDFHLWHL TDFHLWHL TDYHLWHL TDYHLWHL TDYHLWHL TDWHLWHL	GGIAQRGPGE GGIAQRGPGE GHLALKGAGL GGIAQRGPGL GSIAVRGPGI GSFAVRGPAL GSFALRGVPL YATWARGDWG GQFALHGTAL GAMGMRGPGL GQFALHGAAL GGIIQRGPGL	MLIEATAVQP IMVEATAVEP VFIEATAVP MMIEATSVSF TIVESTAVSP TILESIFYSE TIFEATGVLP LILIGNVQVD LILIGNVQVD TIVEATAVSP SMVEATAVSA SMVEATAVQA			QIAPMRQIEPLS TTSEQFLGLKQIAPMKQAEKLKQAHSLRQIAPLK TTPEQTVTAEQIAPLR	RVI-DFVHSQ RVI-EFVHSQ RVI-EFVHSQ RVV-EFMHQQ RVI-DFVHSQ FIV-DYIHSQ RIV-DYIHSQ RIV-EFAHSQ RIV-EFAHSQ RIV-DFIHSQ RIT-TFAHSQ	-5
SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19 SEQ 22 SEQ 24 SEQ 27 SEQ 30 SEQ 33	2	DSS PHVGAL DSS PHVGAL DSS PHVGAL DSS PHVGAL DSS PHVGAL DYNFEA SPTDNQA DOGHM FGNHL DDGHL DDGHL DDGHA CDGGHL CDGGAL CDGGAL	1213 TDYHLAHL TPWHNAHL TNYHLAHL TDYHLHY TDHLHY TDHLHY TDHLWH TDHLWH TDHLWH TDHLWH TDHLWH TDWHLTH TDWHLTH	GGIAQRGPGL GGIAQRGPGF GHIALKGAGI GGIAQRGPGI GSIAVRGPGI GSFALRGVPL YATTARGDWG GQFALHGTAL GAMGWRGPGL GQFALHGAAL GGIIQRGPGL	MLIERTAVQP IMVERTAVEP VFIERTAVOP MHIERTSVSP TIVESTAVSP IILESIFVSE TIFERTGVLP LIITGNVQVD TIVERTSVTP VMVERTAVSP SMVERTAVER SMVERTAVER	E-GRITPQDV E-GRITPQDV E-GRITPQDV E-GRITPQDV E-GGLSPHDL N-SCLSIHDL N-SCLSIHDL N-GRITPECS HAHKGDAHDI N-GRISPEDS E-GRISPNDS R-GRISPEDV H-GRITPQDV		QIAPMRQIEPLS TTSEQFIGLKQIAPMKQAEXLKQAEXLKQAESLRQIAPLK TTPEQTYTAFQIAPLKQIAPLKQIAPLK	RVI-DFVHSQ RVI-EFVHSQ RVI-EFMHSQ RVI-DFVHSQ PIV-DYAHSQ KIV-DFIHSQ KAWADARIN RIV-DYVHSQ RIV-EFAHSQ RIV-EFAHSQ RIV-EFAHSQ RIT-TEAHSQ	-5
SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19 SEQ 22 SEQ 24 SEQ 27 SEQ 30 SEQ 33 SEQ 35	2		1213 TDYHIAHL TPWHPAHL TDYHIAHL TDYHIAHL TPYHLTHY TDWHIWHI TDFLIAN TDFHIWHI TDFHIWHI TDYHIWHL TDYHIWHL TDWHIWHL TDWHIWHL	GGIAQRGPGL GGIAQRGPGL GHLALKGAGL GHLALKGAGL GGIAQRGPGL GSIANRGPGL GSFANRGPAL GAMGHRGPGL GQFALHGTAL GGIIQRGPGL GGFALHGAAL GGIIQRGPGL GGFALHGAAL GGIIQRGPGL	MLIEATAVQP IMVEATAVEP MYTEATAVQP MYTEATAVQP MYTEATAVQP TIVESTAVSP TIFEATGVLP TILESTAVEP VMVEATAVSP VMVEATAVSP SMVEATAVQN TVTEVNAVSP TVTEVNAVSP VMVEATAVON	E-GRITPQDV E-GRITPQDV E-GRITPQDV N-GRISPNDS E-GRITPQDV E-GGLSHDL N-GRITPECS HAHKCDAHDI N-GRISPEDS E-GRISPEDS R-GRISPEDV E-GRISPEDV E-GRISPEDV E-GRISPEDV E-GRISPEDV		QIAPMRQIEPLS TTSEQFLGLKQAEKLKQAEKLKQAEKLKQIAPLK TTPECTVTAFQIAPLKQIAPLKQIEPLK	RVI-DFVHSQ RVV-EFRHAQ RVI-DFVHSQ PIV-DYHSQ PIV-DYHSQ RIV-DFHBQ RIV-DFHSQ RIV-DFHSQ RIV-EFHSQ RIV-EFHSQ RIT-TEAHSQ	-5
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SEO 6										
SEQ 8	LAHAGRKASA	VAPW	-LAAQAGKSS	LKADESVGGW	PADVVGPSGG	EEHIF	SPEEDAYWVP	RA	MTKDDTE	OFKR-DWFDA
SEQ 10	IAHAGRKASN	IAPW	LMNKG	IVATEKVGGW	PDRVIGPSTV	P	EURITEELL	TCDATE	TEKDETK	BAAK-DECTT
SEQ 12	LGHGGRKASG	GVPF	LHLE	QVADKSVNGF	ADKAVAPSAL	P	FSDSHNTP	RE	LIVNEIN	SIVE-DEANA
SEQ 14	LNHAGRKIVE	GVPF		PT-DGDEOGGM	PENVWAPSAI	S	-YNEETFPFP	KE	MTVEQIH	elve-awkas
SEQ 16 SEQ 19	LAHAGRKAST	GAGT	nignons	RGLW	E-KAVAPS PV	P	-LVLGEAFVP	RLLSKVLFGT	PRELTVAEIK	DIV-QKFAVT
SEQ 22	LAHAGRKAST	GAGT KAPWHDSFTP	SGEYKPREGL	QVVGPEYGGW	PDDVWAPSAI	P	FSEDFPNP	KE	MTVEEIE	GLVT-SEVDA
SEQ 24	LAHAGRKAST	KAPWHDSFTP TAPY	RG-Y	TVATEAQGG#	ENDVYGPFTN	E	DRWDENHAQP	HK	ITERQID	PPAD-KEAAN
SEQ 27	LAHAGRKAST	TAPY	ITEARGK	ALAQESENGW	PDDVVAPSAI	P	ITADWATE	KD	FTKEDIE	OLKS-DYVEA
SEQ 30	LSHAGRKASC	VSPW	LSVN	AVAAEEVGGW	PDMIANESMI	A	-20110111111			
SEQ 33		VVPW	TDPV	NTDF?						
SEQ 35 SEQ 38	IGHAGRKAST	VVPW	LSTN	AVAAKEVGGW	PDNIVAPSAI	A	-QEAGVNPVP	KA	FTKEDIE	ELKN-DFLAA
SEQ 40	LINHAGRKAST	VAPW	LSGG	DVAGEDVNGW	PODVWAPSAI	P	WNEKHAVP	KE	MSLDDIE	AFKK-AFGEA EMVQ-DWATA
SEQ 42		VAFW	LPS	KRAGKEAGGW	PEDVVGPSGG	EDFTWDERSS	SDPSGGYYAP	RE	LSVREIK	DATE-CERUT
SEQ 44	VGHPGRQARG	SVQ	QHPISASD	VQLKQEM			LODITIONE	D.B	TOTEFTE	OT KE-DEVSG
SEQ 83	IGHAGRKASC	SVQ VAPW	LDAG	LAAEKAAGGW	PDDVVGPSNE	P		RA	LTTEDIN	KLOD-KEVOS
SEQ 85	LAHAGRKASD	WSPF	YRGEKKQ	KEVTQEEGGW	PDKAAVEDWT	A	27 Igoni van			
Bacteria		NRPW	FGDD	HIGHDDARGW	ETTAPSAI	A	FGAHLPNV	PRA	MTLDDIA	RVKQ-DFVDA
T44612	IAHAGRKASA	NRPW	BGG	APVGADAYGW	OPLAPSAL	A	FDERHPVP	TE	LTVPQIQ	EAVG-READA GVVD-AESAA
NP_625402 NP 295913	IMMAGRAMAT	YAPW	RGK	GAVPAELGGW	QVIGPDEN	S	FHDLFPTP	AM	MGADELR	GVVD-AFSAA RVRN-DEVRA
AF320254										
OYE family		KLAW						77	TTEDETO	OCTA-DEAGA
Af4875	LWATGRAADP	DVLA	DMKD	LISSS-AVPV	EEKGP			TE	ITTP-HL	KOTIRDYCNA
Af4961	LWHAGRATIP	QMTG	spavsas	ATVWDSPTEC	YSHPP	VGST	KTAKSVGNEL	RE	LTEKEID	HIVEVEYPNA ERVA-EYAAA
Ca2460	LWYLGRVANP	KDLK	DAGDED	IGESAVIN	DEE35			FF	MTVAEIK	ERVA-EYAAA
Nc4452	LWSLGRAANP	EVLA	REGGER	YDSASDNVEM	DAEOE		AKAKKANNPQ	HS	LTKDEIK	QYIK-EYVQA QYVK-EYVQA
ScOYE1 ScOYE2	INVICUA A FP	DTLA	RDG-LR	YDSASDNVYM	NAEQE		EKAKKANNPQ	'HS	ITKDEIK	QYVK-EYVQA QYIK-DYIHA
SCOYES	LWSLGWASEP	DVLA	RDG-LR	YDCASDRVYM	NATLQ		EKAKDANNLE	HS	LTKDDIK	QYIK-DYIHA HTVEVEYPNA
A36990	LWYLGRVANA	KDLK	DSG-LP	LIAPS-AVYW	DENSE		KLAKEAGNEL	ка	DIEFETD	HIVEVEYPNA
			201	221	341	35İ	361	371	381	391
	301	311			341				8	
									8	
SEO 3							DAYGRAYA	VFLR	8 TSAS-DWCE-	ETLPEQ
SEQ 3 SEQ 6	TKRAIAAGA VKRAVKAGA	DFVEIHNAHG DFIEIHNAHG	YLLSSFLSP-	-AANNRTDQY -AVNTRTDEY	G-GSFENRIR G-GSFENRIR	LSLEIAQLTR	DAVGPHVP	VFLR	ISAS-DWCE- VSAT-DWLE-	ETLPEQEVQPNKP
	TKRAIAA-GA VKRAVKA-GA ARIAVQA-GV	DFVEIHNAHG DFIEIHNAHG DVIEIHGAHG	YLLSSFLSP- YLLMSFLSP- YLINEFLSP-	-AANNRTDQY -AVNTRTDEY -VTNKRTDAY	G-GSFENRIR G-GSFENRIR G-GSFENRTR	LSLEIAGLTR LSLEIAKLTR IVREVAAAIR	DAVGPHVP ENVPKDMP AVIPEGMP	VFLR	ISAS-DWCE- VSAT-DWLE- ISAT-EWLE- VSAT-DWIE-	ETLPEQEVQPNKP -GQPVAAESG
SEQ 6 SEQ 8 SEQ 10	TKRAIAA-GA VKRAVKA-GA ARIAVQA-GV CKRAIAA-GA	DFVEIHNAHG DFIEIHNAHG DVIEIHGAHG DFIEIHNAHG	YLLSSFLSP- YLLMSFLSP- YLINEFLSP- YLLSSFLSP-	-AANNRTDQY -AVNTRTDEY -VTNKRTDAY -SSNTRTDEY	G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR	LSLEIAQLTR LSLEIAKLTR IVREVAAAIR LSLEIAQVTR	DAVGPHVP ENVPKDMP AVIPEGMP DAVGPNVP	VFLRVFLRVFLR	ISAS-DWCE- VSAT-DWLE- ISAT-EWLE- VSAT-DWIE- ISAB-ENSP-	ETLPEQEVQPNKP -GQPVARESGETLPEE
SEQ 6 SEQ 8 SEQ 10 SEQ 12	TKRAIAA-GA VKRAVKA-GA ARLAVQA-GV CKRAIAA-GA ARRAVEISGF	DFVEIHNAHG DFIEIHNAHG DVIEIHGAHG DFIEIHNAHG DAVEIHGAHG	YLLSSFLSP- YLIMSFLSP- YLINEFLSP- YLLSSFLSP- YLINEFYSP-	-AANNRTDQY -AVNTRTDEY -VTNKRTDAY -SSNTRTDEY -ISNKRTDEY	G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR	LSLEIAQLTR LSLEIAKLTR IVREVAAAIR LSLEIAQVTR FLKEVIDSVK	DAVGPHVP ENVPKDMP	VFLRVFLRVFLRVFLR	ISAS-DWCE- VSAT-DWLE- ISAT-EWLE- VSAT-DWIE- ISAA-ENSP- FPMS-DNCS-	ETLPEQEVQPNKP -GQPVAAESGETLPEE
SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14	TKRAIAA-GA VKRAVKA-GA ARIAVQA-GV CKRAIAA-GA ARRAVEISGF AWRAVEISKF	DFVEIHNAHG DFIEIHNAHG DVIEIHGAHG DFIEIHNAHG DAVEIHGAHG DAVEIHGAHG	YLLSSFLSP- YLLMSFLSP- YLINEFLSP- YLLSFLSP- YLINEFYSP- CLIHQFLSK-	-AANNRTDQY -AVNTRTDEY -VTNKRTDAY -SSNTRTDEY -ISNKRTDEY -ITNKRADQY	G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRVR	LSLEIAQLTR LSLEIAKLTR IVREVAAAIR LSLEIAQVTR FLKEVIDSVK FILQIIENIK	DAVGPHVP ENVPKDMP AVIPEGMP DAVGPNVP SSIPNDVP RVIETP	VFLRVFLRVFLRVFLRVFLR	ISAS-DWCE- VSAT-DWLE- ISAT-EWLE- VSAT-DWIE- ISAA-ENSP- FPMS-DNCS- VSAT-EWME-	ETLPEQEVQPNKP -GQPVAAESGETLPEEDPEDPE
SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16	TKRAIAA-GA VKRAVKA-GA ARIAVQA-GV CKRAIAA-GA ARRAVEISGF AWRAVEISKF AQRALKA-GF	DFVEIHNAHG DFIETHNAHG DVIETHGAHG DFIETHNAHG DAVETHGAHG DATETHCANG DATETHCANG	YLLSSFLSP- YLLMSFLSP- YLLMSFLSP- YLLSSFLSP- YLIMSFYSP- CLIHQFLSK- YLISEFLSP-	-AANNRTDQY -AVNTRTDEY -VTNKRTDAY -SSNRTRDEY -ISNKRTDEY -ISNKRTDEY -LINKRADQY	G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRVR G-GSFENRVR	LSLEIAQLTR LSLEIAKLTR IVREVAAAIR LSLEIAQVTR FLKEVIDSVK FLLQIIENIK VLREIISAVR	DAVGPHVP ENVPKDMP AVIPEGMP DAVGENVP SSIPNDVP RKIET SVIPEDMP	VFLRVFLRVFLRVFLRVFLR	ISAS-DWCE- VSAT-DWLE- ISAT-EWLE- VSAT-DWIE- ISAA-ENSP- FPMS-DNCS- VSAT-EWME- INSA-DWOA-	ETLPEQEVQPNKP -GQPVAAESGETLPEEDPEYTGQPGRDGKEEEE
SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19	TKRAIAA-GA VKRAVKA-GA ARIAVQA-GV CKRAIAA-GA ARRAVEISGF AWRAVEISGF AQRAIKA-GF AQRAIKA-GF ARIIAEA-GF	DFVEIHNAHG DFIEIHNAHG DVIEIHGAHG DFIEIHNAHG DAVEIHGAHG DAIEIHCANG DLIEIHAAHG NGVEIHAAHG	YLLSSFLSP- YLIMSFLSP- YLIMSFLSP- YLLSSFLSP- YLIMSFYSP- CLIHQFLSK- YLISEFLSP- YLLAQFLSK-	-AANNRTDQY -AVHTRTDEY -VTNKRTDAY -SSNTRTDEY -ISNKRTDEY -ISNKRADQY -ISNCRTDQY -KTNRRGDEY	G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRVR G-GSFENRVR G-GSFENRVR	LSLEIAQLTR LSLEIAKLTR IVREVAAAIR LSLEIAQVTR FLKEVIDSVK FLLQIIENIK VLREIISAVR IVGEIIKECR	DAVGPHVP— ENVPKDMP— AVIPEGMP— DAVGENVP— SSIPNDVP— RKIET—P— SVIPEDMP— RQVTEAVGEE	VFLRVFLRVFLRVFLR		ETLPEQEVQPNKP -GQPVAAESGETLPEEDPEYTGQPGRDGKEEEE
SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16	TKRAIAA-GA VKRAVKA-GA ARIAVQA-GV CKRAIAA-GA ARRAVEISGF AWRAVEISKF AQRAIKA-GF ARIIAEA-GV	DFVEIHNAHG DFIEIHNAHG DFIEIHNAHG DFIEIHNAHG DAVEIHGAHG DAIEIHCANG DLIEIHAAHG NGVEIHAAHG DIIEIHAAHG	YLLSSFLSP- YLIMSFLSP- YLIMEFLSP- YLLSSFLSP- YLLMEFYSP- CLIHQFLSK- YLISEFLSP- YLLAQFLSK- YLITEFLSP-	-AANNRTDQY -AVNTRTDEY -VTNKRTDAY -SSNTRTDEY -ISNKRTDEY -LINKRADQY -ISNGRTDQY -KTNRRGDEY -LSNKRTDKY	G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR	LSLEIAQLTR LSLEIAKITR IVREVAAAIR LSLEIAQVTR FLKEVIDSVK FLLQIIENIK VLREIISAVR IVGEIIKECR VLIDIIKAVR	DAVGPHVP ENVPKDMP AVIPEGMP BSIPNDVP RKIET RQVTEAVGEE AVIPEEM RYIPESMP	VFLRVFLRVFLRVFLRVFLRIFLKIFLK		ETLPEQEVQPNKP -GQPVAAESG
SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19 SEQ 22 SEQ 24 SEQ 27	TKRAIAA-GA VKRAVKA-GA ARIAVQA-GV CKRAIAA-GA ARRAVEISGF AWRAVEISKF AQRALKA-GF AKRAIEA-GF AKRAIEA-GF AKRAVEI-GF AKRAVEI-GF	DFVEIHNAHG DFIEIHNAHG DFIEIHNAHG DFIEIHNAHG DAVEIHGAHG DAVEIHGAHG DLIEIHAAHG NGVEIHAAHG DVIEIHGAHG DVIEIHGAHG	YLLSSFLSP- YLLMSFLSP- YLLMSFLSP- YLLSFLSP- YLINEFYSP- CLIHQFLSK- YLISEFLSP- YLLAQFLSK- YLITEFLSP- YLISSTVSPA	-AANNRTDQY -AVNTRTDAY -SSNTRTDAY -SSNTRTDAY -ISNKRTDAY -ISNKRTDAY -ISNGRTDQY -KTNRRGDAY -KTNRRGDAY -KTNRRGDAY	G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRVR G-GSFENRVR G-GSFENRTR G-GSFENRTR G-GSFENRTR G-GSFENRTR	LSLEIAQLTR LSLEIAKLTR IVREVAAAIR LSLEIAQVTR FLKEVIDSVK FLLQIIENIK VLREIISAVR IVGEIIKECR VLIDIIKAVR FPMEVVHSVR	DAVGPHVP ENVPKDMP AVIPEGMP DAVGENVP SSIPNDVP RKIETP SVIPEDMP RQVTEAVGBE AVIPEEM KAIPDSMP	VFLRVFLRVFLRVFLRVFLR	ISAS-DWCE- VSAT-DWLE- VSAT-DWLE- VSAT-DWIE- ISAA-ENSP- FPMS-DNCS- VSAT-EWME- INSA-DWQA- ISAT-EWME- VTAT-DWLP-	ETLPEQEVQPNKP -GQPVAAESGETLPEEDPEYTGQPGADGKEEEEYAGEP
SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19 SEQ 22 SEQ 24 SEQ 27 SEQ 30	TKRAIAA-GA VKRAVKA-GA ARIAVQA-GV CKRAIAA-GA ARRAVEISGF AWRAVEISKF AQRALKA-GF AKRAIEA-GF AKRAIEA-GF AKRAVEI-GF AKRAVEI-GF	DFVEIHNAHG DFIEIHNAHG DFIEIHNAHG DFIEIHNAHG DAVEIHGAHG DAVEIHGAHG DLIEIHAAHG NGVEIHAAHG DVIEIHGAHG DVIEIHGAHG	YLLSFLSP- YLIMSFLSP- YLIMSFLSP- YLIMSFLSP- YLIMSFYSP- CLIMGFLSK- YLISEPLSP- YLLAGFLSK- YLITEFLSP- YLISSTVSPA YLLHQFLSP-	-AANNRTDQY -AVNTRTDEY -VINKRTDAY -SSNRRTDEY -ISNKRTDEY -ISNKRTDEY -ISNRRTDEY -ISNRRTDEY -ISNRRTDEY -ISNRRTDEY -TNDRNDKY -VSNQRTDEY	G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRVR G-GSFENRVR G-GSFENRTR G-GSFENRIR G-GSFENRIR G-GSFENRIR	LSLEIAQLTR LSLEIAKUTR IVREVARAIR ESLEIAQVTR FIKEVIDSVK FILQIIENIK VIREIISAVR IVGEIIKECR VIJDIIKAVR FFMEVVHSVR	DAVGPHVP— ENVPKDMP— AVIPEGMP— DAVGPNVP— SSIPNDVP— RKIET——P— RVIPEDMP— RQVTEAVGEE AVIPEEM— KAIPDSMP—	VFLRVFLRVFLRVFLRIFLKIFVR EAKKFVVGIK	ISAS-DWCE- VSAT-DWLE- ISAT-EWLE- VSAT-DWIE- ISAN-ENSP- FFMS-DNCS- VSAT-EWME- LNSA-DWQA- ISAT-DWLE- VSAT-DWLE-	ETLPEQEVQPNKPGQVARESG
SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19 SEQ 22 SEQ 24 SEQ 27 SEQ 30 SEQ 33	TKRATAA-GA VKRAVKA-GA ARLAVQA-GV CKRATAA-GA ARRAVEISGF AWRAVEISGF AWRAVEISGF ARRALEA-GF ARRAIEA-GF AKRAIEA-GF AKRAYEI-GF AKRAYEI-GF AKRAYEI-GF AKRAYEI-GF	DEVEINANG DEVEINANG DEVEINANG DEVEINANG DEVEINANG DAVEINANG DAVEINANG DAVEINANG DUIETHAANG DUIETHAANG DVIETHAANG DVIETHAANG DVIETHAANG DVIETHAANG	YLLSFLSP- YLLMSFLSP- YLIMSFLSP- YLLSSFLSP- YLLSSFLSP- YLISEFLSP- YLISEFLSP- YLLAGFLSK- YLITEFLSP- YLLMGFLSP- YLLMGFLSP-	-AANNRTDQY -AVNTRTDBY -VTNKRTDAY -VSNKRTDEY -ISNKRTDEY -ISNKRADQY -ISNKRADQY -ISNKRTDKY -KTNRGDBY -LSNKRTDKY -LSNKRTDKY -TTNDRNDKY -VSNQRTDEY	G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRUR G-GSFENRUR G-GSFENRUR G-GSFENRUR G-GSFENRUR G-GSFENRUR G-GSFENRUR G-GSFENRUR G-GSFENRUR	LSLEIAQLTR LSLEIAKLTR LYREVAAAIR LSLEIAQVTR FIKEVIDSVK FILQIIENIK VLREIISAVR IVGEIIKAV VLIDIIKAV FPMEVVHSVR	DAVGPHVP— ENVPKDMP— AVIPEGMP— DAVGENVP— SSIPNDVP— SVIPEDMP— RQVTEAVGES AVIPEEM— KAIPDSMP—	VFLR	ISAS-DWCE- VSAT-DWLE- ISAT-EWLE- VSAT-DWIE- ISAT-EWNE- VSAT-DWIE- ISAT-EWNE- LNSA-DWQA- ISAT-EWNE- VTAT-DWLP- VSAT-DWFEE	ETLPEQEVQENKFGQPVARESGDPEDPEYAGEPKGQ
SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19 SEQ 22 SEQ 24 SEQ 27 SEQ 30 SEQ 33 SEQ 33 SEQ 35	TKRAIAA-GA VKRAVKA-GA ARIAVQA-GV CKRAIAA-GA ARRAVEISKF AQRAIKA-GF ARIITAEA-GF AKRAIEA-GV AKRAVEI-GF AKRAIHA-GF	DEVEINANG DETEINANG DETEINANG DVIEINGANG DATEINANG DATEINANG DATEINANG DIETHANG DIETHANG DVIEINANG DVIEINANG DVIEINANG DVIEINANG	YLLSSFLSP- YLLMSFLSP- YLIMEFLSP- YLLSSFLSP- YLLSSFLSP- YLLSSFLSP- YLLSSFLSP- YLLAGPLSK- YLITEFLSP- YLLSSTVSFA YLLSSTVSFA	-AANNRTDQY -AVITATDEY -VTNKRTDAY -SSNTRTDBY -ISNKRTDBY -ISNKRTDBY -ISNKRTDBY -ISNKRTDBY -ISNKRTDBY -ISNKRTDKY -ISNKRTDKY -ISNKRTDKY -ISNKRTDKY -ISNKRTDKY -ISNKRTDKY -TDRNKKY -VSNQRTDEY	G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRUR	LSLEIAQLTR LSLEIAKUTR LSLEIAQVTR LSLEIAQVTR FLKEVIDSVK FLLQIIENIK VLREIISAVR IVGEIIKECR VLIDIIKAVR FPMEVVHSVR	DAVGPHVP- ENVPKDMP- AVIPEGMP- DAVGENVP- SSIPNDVP- SVIPEDMP- RQVTEAVGEE AVIPEEM- KAIPDSMP- AAIPETTP-	VFLRVFLRVFLR	ISAS-DWCE- VSAT-DWLE- ISAT-EWLE- VSAT-DWIE- ISAT-EWIE- ISAA-ENSP- FPMS-DNCS- VSAT-EWME- LINSA-DWG- ISAT-EWME- VTAT-DWLP- VSAT-DWFEE	ETLPEQEVQPNKP
SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19 SEQ 22 SEQ 24 SEQ 27 SEQ 30 SEQ 33 SEQ 35 SEQ 38	TKRAIRA-GA VKRAVKA-GA ARIAVQA-GV CKRAIRA-GA ARRAVEISGF AWRAVEISKF AQRAIKA-GF AKRATERA-GF AKRATERA-GF AKRATERA-GF AKRATHA-GF AKRATHA-GF AKRATHA-GF AKRATHA-GF	DEVEINANG DEVEINANG DEVEINANG DEVIETHGANG DEVEINANG DAVELHGANG DAVELHGANG DAIEHAANG DIJEHAANG DIJEHAANG DVIETHGANG DVIETHAANG DVIETHAANG DVIETHAANG DVIETHAANG	YLLSFLSP- YLLMSFLSP- YLIMSFLSP- YLIMSFLSP- YLIMSFLSK- YLIMSFLSK- YLLSFLSK- YLLMSFLSK- YLLMSFLSK- YLLMSFLSK- YLLMSFLSK- YLLMSFLSF- YLLMSFLSF- YLLMSFLSF- YLLMSFLSF-	-AANNRTDCY -AVITRIDEY -AVITRIDEY -VINKRTDAY -SSNIPRIDEY -ISNKRTDEY -ISNKRTDEY -ISNKRTDEY -ISNKRTDEY -ISNKRTDEY -TINKRADGY -TINKRTDEY -VSNQRTDEY -VSNQRTDEY -VSNQRTDEY	G-GSPENRIR G-GSPENRIR G-GSPENRIR G-GSPENRIR G-GSPENRIR G-GSPENRIR G-GSPENRIR G-GSPENRIR G-GSPENRIR G-GSPENRIR G-GSPENRIR	LSLEIAQLTR LSLEIAKLTR LVREVAAAIR LSLEIAQVTR FILKEVIDSVK FILQUIENIK VLREIISAVR IVGEIIKECR VLIDIIKAVR FPMEVVHSVR VVLEILDLIR VVLEILDLIR	DAVGPHVP— ENVPKDMP— AVIPEGMP— DAVGPNVP— SSIPMDVP— SVIPEDMP— RQVTEAVGBE KAIPDSMP— KAIPDSMP— AAIPETTP—	VFLRVELRVELRVELR	ISAS-DWCE- VSAT-DWLE- ISAT-EWLE- VSAT-DWIE- ISAA-ENSP- VSAT-DWCS- VSAT-EWME- LINSA-DWQA- ISAT-EWME- VTAT-DWLP- VSAT-DWEEE	ETLPEQEVQPINEEVQPINE
SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19 SEQ 22 SEQ 24 SEQ 27 SEQ 30 SEQ 33 SEQ 35 SEQ 38 SEQ 38	TKRAIRA-GA VKRAVKA-GA RALAVQA-GV CKRAIRA-GA ARRAVEISKF AQRALKA-GF AKRAIEA-GF AKRAIEA-GF AKRAIEA-GF AKRAIHA-GG	DEVEIHNAHG DFISTHNAHG DFISTHNAHG DVIETHGAHG DAVETHGAHG DAVETHCANG DLIETHAAHG DLIETHAAHG DVIETHAAHG DVIETHAAHG DVIETHAAHG DVIETHAAHG DVIETHAAHG DVIETHAAHG	YLLSFLSP- YLLMSFLSP- YLIMSFLSP- YLLNEFLSP- YLLNEFYSP- CLINGFLSK- YLISFLSF- YLLAGPLSK- YLIAGPLSK- YLITFLSP- YLLHGFLSP- YLHQFLSP- YLHQFLSP- YLHQFLSP-	-AANNRTDQY -AVITRTDBY -VINKRTDAY -SSNTRTDBY -ISNKRTDBY -ISNKRTDEY -ISNKRTDEY -ISNKRTDEY -ISNKRTDEY -ISNKRTDEY -ISNKRTDEY -ISNKRTDEY -ISNKRTDEY -ISNKRTDEY -VSNQRTDEY -VSNQRTDEY -VSNQRTDEY -VSNQRTDEY -RATEGPIST	G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR	LSLEIAGLTR LSLEIAKLTR LSLEIAGVTR LSLEIAGVTR FLIGEIIGNIK VIREIISAVR FLIGEIIKECR VILDIIKAVR FFMEVVHSVR VVLEILDLTR VVLEILDLTR VVLEII— LTMESRRPCE	DAVGPHYP- ENVPKDMP- AVIPEGMP- DAVGENVP- SSIPNDVP- RKIET-P- SVIPEDMP- RQVTEAVGEE AVIPEEM- KAIPDSMP- AAIPETTP-	VFLRVELRVELR	ISAS-DWCE- VSAT-DWLE- ISAT-EWLE- VSAT-DWIE- ISAA-ENS- FFMS-DNCS- VSAT-EWME- VTAT-DWLP- VSAT-DWLP- VSAT-DWEE- VSAT-DWEE- USSA-DWEE- VSAT-DWEE-	ETLPEQEYQPIKE -GQPVAAESGETLPEL
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SEO 6 SEO 10 SEO 12 SEO 14 SEO 12 SEO 14 SEO 15 SEO 22 SEO 27 SEO 30 SEO 35 SEO 35 SEO 44 SEO 93 SEO 45 SEO 45 SEO 47 SEO 15 Bacteria T4612 NP_255913 Af 320251 OTE family Af 387 Af 386 Nc 4452 SCOYEL	TKRAINA-GA ARIAVICA-GA ARIAVICA-GA ARIAVOA-GV CKRAINA-GA ARRAVEISEF ARIATEA-GV AKRAIEA-GV AKRAIEA-GV AKRAIHA-GE	DEVELHNANG DPISTHNANG DPISTHNANG DPISTHNANG DPISTHNANG DPISTHNANG DPISTHNANG DPISTHNANG DAVEIHANG NEVETHANG DILITHANG DILITHANG DILITHANG DVIETHANG DILBERCANG DRYETHANG DVIETHANG DVIETHA	YLLSSFLSP- YLLMSFLSP- YLIMSFLSP- YLIMSFLSP- YLIMSFLSP- YLIMSFLSS-	-AANNRTDOY -ANNRTDEY -VINKRTDAY -VINKRTDAY -ISNRATDEY -ISNRATDEY -ISNRATDOY -ISNRATDOY -ISNRATDOY -ISNRATDOY -VINCRTDEY -VINCRTDEY -VINCRTDEY -VINCRTDEY -VINCRTDEY -VINCRTDEY -VINCRTDEY -VINCRTDEY -ITNRATDEY -INNRATDAY -INNRATDAY -INNRATDEY -	G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR G-GSFENRIR	LSLEIAQUTR LSLEIAQUTR LSLEIAQUTR LSLEIAQVTR FLKEVIDSVK VLREIISAVR FPHEVVHSVR VVLEIISAVR VVLEILDLIR LTMESRREGE LLIELVTAVR LLIELVTAVR LLIELVTAVR FLLEVTAVR ELLIELVTAVR FLLEVTAVR LLLELVTAVR FLLEVEARR FLLEVARAVR FLLETLANGE FALEVURAVR LLIELVRAVR FLLEVLAVR FALEVURAVR LLIELVRAVR FLLEVLAVR FALEVURAVR FALEVURAVR FAVEVERAVI FAVEVERAVI FAVEVERAVI FALEVURAVR FALEVURAVA FATEVURAVAV FTLEVURAVA FTLEVURAVAV FTLEVURAVAV FTLEVURAVAV FTLEVURAVAV FTLEVURAVAV FTLEVURAVAV FTLEVURAVAV FTLEVURAVAV FTLEVURAVAV FTLEVURAVAV FTLEVURAVAV FTLEVURAVAV FTLEVURAVAV FTLEVURAVAV FTLEVURAVAV FTLEVURAVAV FTLEVURAVAV FTLEVURAVAV FTLEVURAVAV FTLEVURAVAV	DAVGPHVP- ENVPKDMP- AVIPEGMP- AVIPEGMP- SSIPHDVP- SSIPHDVP- SSIPHDVP- RQVTEAVGBE- KAIPDSMP- AAIPETTP- AAIPETTP- AAIPETTP- CH7- AAMPSSMP- RRTSKNF- EWMPDIMP- CEFPKKG- EWMPDIMP- EWMPDIKP- HWPAILLP- AAWPTMP- EAWGADR- ATVGEDN- GTVGABK- EAWGABK- EAW		ISAS-DWCE- ISAS-DWCE- ISAS-DWCE- ISAT-EWE- ISAS-EWE- ISA	DSQKMEFFE

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SEQ 3 .										
SEQ 6	SWRGVDTVR-	FAQELVKQ -FAKILA-ET	GYVDVLDVSS	GGTHSEQ	нт	HAKPGFQAPF	ALAVKNAVGD	KLAVASV	THERMSTAND	SECRECTAGE
SEQ 8	SWDM-QSSL-	-FAKILA-ET ELVKKLPE FAEALAAQ	WGIDLVDVSS	AANHKDQ	KI	NUMTATOTOL	AGGIROAI	KTJVRTV	GTIT	
SEQ 10	SWKLSDSVR-	FAEALAAQ	GAIDLIDVSS	GGVHAAQ	DOCTOR	PIDEDIUMET.	CONTROPUED	KT-T-VSCV	GGLE	
SEQ 12										
SEQ 14										
SEQ 16										
SEQ 19	TDTAEEVLK-	-QIELFEQ -LAKLLPD TLAARLRD	T.CVDT.T.DVSS	GENSVAO	КІ	ELTPYYOIDL	AAKIREAVGD	RLLIGAV	GNIN	~~
SEQ 22 SEO 24	PADTE 6216-	TIARDIAD	CGVDLTDVSS	GGNHKDO	RI	EVKDCYQVPF	AEKIKDQVNG	ILLGAV	GMIR	
SEQ 27	GREADIVAE	TLAARLRD								
SEQ 30										
SEQ 33										
SEO 35										
SEQ 38										
SEQ 40						<u></u>				
SEQ 42	SWDVESTIK-	-ISKILAD LCEALEAAGM	LGVDLLDVSS	GGNHPQQ	KI	NMENT	PENTENDINA	M///YTTG	GEKT	
SEQ 44	FKP-EEAVQ-	LCEALEAAGM -LAHQLAD LAKMLQE	DFVETSG	GTYESFG	FARRESS	ANGEOGYODES.	VEATKKAAU	KMLTSTV	GSIK	
SEQ 83	TWTLEQSIK-	-LAHQLAD	RGVDVLDVSS	GGIHRMQ	KT	TVGDGYOLFG	AKAVRDATAK	TEPDASKR	MLVGA	
SEQ 85	SWTVDQTVE-	LAKMLQE	ARVULUUVSS	GGHVELQ						
Bacteria										
T44612 NP 625402	EQTLEESI	ELARREKA -FARDLEA	HGTDLLDVST	GGNVPRV	RI	PTGPGYQVPF	AARVKAGST-	LPVAAV	GLIT	
NP_625402 NP 295913										
AF320254	CHIDDENT VA-	-LSKLLKY -TARLFKE	AGADIIDOSS	GOVWKGD	QP	VYGRMYQTPF	ADRIRNEVGI	PTLAVG	AISE	
OYE family										
Af4875										
A£4961	EQR-VETWTF	LCESLKKAHP	NLSYVSF	IEPRYE		-QIFSYEEKD	NFLRSWG	LSDVDLSSFR	KIEGETPEES	
Ca2460										
Nc4452										
ScOYE1	ETGIVAQYAY	VAGELEKRAK	AGKRLAFVHL	VEPRVING	E DIEGE	GETEGGSNUE	NYSTWKG	PTTRA	GNEA	
SCOYE2										
SCOYE3	EPGIIAQYSY	VLGELEKRAK ILQQLQQRAD	AGKRLAEVHL	VERRVIDE	TYDVST	KDOOGRSNEF	AYKIWKG	NEIRA	GNYT	
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A36990							5.61	571	581.	591
A36990	501	511	521	531	541	551	561	571	581	591 1
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SEQ 3	501NGKQANSAHLANS ADEATAREAM	511 QILEEQD LLEKDG LSGPEPK	521	531	541IDVALVGIDIVLVG	SS1 RGFQKDPGLA RGFQKNPGLV RQFLREPEWV	561 WTFAQHLGV- WAWADELNV- FSTARKLGV-	571	581 	591 1 QIRWGFTRRG QIRWGFSRRG QFGRAI OTBWGFTRRG
SEQ 3 SEQ 6 SEQ 8 SEQ 10	501NGKQANSAHLANS ADEATAREAM	511 QILEEQD LLEKDG LSGPEPK	521	531	541IDVALVGIDIVLVG	SS1 RGFQKDPGLA RGFQKNPGLV RQFLREPEWV	561 WTFAQHLGV- WAWADELNV- FSTARKLGV-	571	581 	591 1 QIRWGFTRRG QIRWGFSRRG QFGRAI OTBWGFTRRG
SEQ 3 SEQ 6 SEQ 8 SEQ 10 SEQ 12	501	511 QILEEQD LLEKDG KLLEEG KYLEEGT	521	531	541IDVALVGIDLVLVGADAILIAIDVALVGFDIALIG	RGFQKDFGLA RGFQKNPGLV RQFLREPEWV RGFQKDPGLA RGFLRNPGLV	TFAQHLGV- WAWADELNV- FSTARKLGV- WTFAQHLDV- WEFADKLGV-	571	5811EISMANEISMANEISMAN	591 1 VIRWGFTRRG QIRWGFTRRG QFGRAI QIRWGFTRRG QLGWGFWPNK QVKLALS
SEQ 3 SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14	501NGKQANSAHLANS ADEATAAEAMNGKQANKDPELLN	OILEEQD LLEKDG LSGFEPK KLLEEGG EFIANGD	521	531	541	RGFQKDFGLA RGFQKNPGLV RQFFLREPEU RGFQKDFGLA RGFLRNPGLV KGFLKNTGLI	WTFAQHLGV- WAWADELNV- FSTARKLGV- WTFAQHLDV- WEFADKLGV- SRIADQLQA-	571	5811EISMANEISMANEIAMAS	591 1 QIRWGFTRRG QIRWGFSRRG QFGRAI QIRWGFTRRG QLGWGFWPNK QYKLALS
SEQ 3 SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16	501NGKQANSAHLANS ADEATAAEAMNGKQANKDPELLN	OILEEQD LLEKDG LSGFEPK KLLEEGG EFIANGD	521	531	541	RGFQKDFGLA RGFQKNPGLV RQFFLREPEU RGFQKDFGLA RGFLRNPGLV KGFLKNTGLI	WTFAQHLGV- WAWADELNV- FSTARKLGV- WTFAQHLDV- WEFADKLGV- SRIADQLQA-	571	5811EISMANEISMANEIAMAS	591 1 QIRWGFTRRG QIRWGFSRRG QFGRAI QIRWGFTRRG QLGWGFWPNK QYKLALS
SEQ 3 SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19	501NGKQANSAHLANS ADEATAAEAMNGKQANKDPELLN	OILEEQD LLEKDG LSGFEPK KLLEEGG EFIANGD	521	531	541	RGFQKDFGLA RGFQKNPGLV RQFFLREPEU RGFQKDFGLA RGFLRNPGLV KGFLKNTGLI	WTFAQHLGV- WAWADELNV- FSTARKLGV- WTFAQHLDV- WEFADKLGV- SRIADQLQA-	571	5811EISMANEISMANEIAMAS	591 1 QIRWGFTRRG QIRWGFSRRG QFGRAI QIRWGFTRRG QLGWGFWPNK QYKLALS
SEQ 3 SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19 SEQ 22	501NGKQANSAHLANS ADEATAAEAMNGKQANKDPELLNRDIFKLD E-DGRVTIQRTRQGMETADIAR	OILEEQD LIEKDG LSGPEPK KILEEGG EFIANGD ENGAKTR AALESDD DVVDEQGAEK	521	531	541	RGFQKDFGLA RGFQKNPGLV RGFLRE PEWV RGFQKDPGLA RGFLRNEGLV KGFLKNTGLI RQFLKE PEFV RPAIINPSLE RQFLRE PEFV	WTFAGHLGV- WAWADELNV- FSTARKLGV- WTFAQHLDV- WEFADKLGV- SRIADQLQA- LTVADELGV- ANLILMPEV- LRTAHNLGV-	571	5811	291 1 QIRWGFTRRG QIRWGFSRRG QIRWGFTRRG QIRWGFWPNK QYKLALS QYIRGPLSSR LFFKKRAEPH QYIRRAVWRKG
SEQ 3 SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19 SEQ 22 SEQ 24	501NGKQANSAHLANS ADEATAAEAMNGKQANKDPELLNRDIFKLD E-DGRVTIQRTRQGMETADIAR	OILEEQD LIEKDG LSGPEPK KILEEGG EFIANGD ENGAKTR AALESDD DVVDEQGAEK	521	531	541	RGFQKDFGLA RGFQKNPGLV RGFLRE PEWV RGFQKDPGLA RGFLRNEGLV KGFLKNTGLI RQFLKE PEFV RPAIINPSLE RQFLRE PEFV	WTFAGHLGV- WAWADELNV- FSTARKLGV- WTFAQHLDV- WEFADKLGV- SRIADQLQA- LTVADELGV- ANLILMPEV- LRTAHNLGV-	571	5811	291 1 QIRWGFTRRG QIRWGFSRRG QIRWGFTRRG QIRWGFWPNK QYKLALS QYIRGPLSSR LFFKKRAEPH QYIRRAVWRKG
SEQ 3 SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19 SEQ 22 SEQ 24 SEQ 27		QILEEQD LIEKDG LSGPPK KYLEEGG KYLEEGG EFIANGD ENGAKTR PALESDD DVVDEQGAEK EILESGK	521	531	541	RGFQKDPGLA RGFQKNPGLV RQFLKEPEWV RGFLKNPGLV KGFLKNFGLV KGFLKEPEFV RPAIINPSLE RQFLKEPEFV REFLKNPSLV	WTFAQHLGV- WAWADELNV- FSTARKLGV- WTFAQHLDV- WEFADKLGV- SRIADQLQA- LTVADELGV- ANLILNPEV- LRTAHNLGV- LDSANQLGE-	571	581	591 1 QIRWGFTRRG QIRWGFSRRG QIFWGFWPNK QYKIALS QYINGFUSR LFDKKRAEPH QYINRAVWRKG QYDYROVKGHR
SEQ 3 SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 19 SEQ 22 SEQ 24 SEQ 27 SEQ 27 SEQ 30		GILEEQD	521 VAEAKQTHDT	531	541	RGFQKDPGLA RGFQKDPGLA RGFQKDPGLA RGFLKEPERV RGFLKNTGLI RQFLKEPERV RPAIINESLV RQFLREPERV REFLENPSLV	WTFAQHLGV- WAWADELNY- FSTARKLGV- WTFAQHLDV- WEFADKLGV- SRIADQLQA- LTVADELGV- ANLINDEV- LRTAHNLGV- LDSANQLGE-	571	581	591 1 QIRWGFTRRG QIRWGFTRRG QIRWGFTRRG QIRWGFTRRG QLGWGFWPNK QYKLALS QYLRGPLSSR QYDRAVKGHR QYDRAVKGHR
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SEQ 3 SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 16 SEQ 22 SEQ 24 SEQ 27 SEQ 23 SEQ 33 SEQ 33 SEQ 35		GILEEQD	521	531	541IDVALVGIDIVINGADAILIALDVALVG	RGFQKDPGLA RGFQKPGLV RGFQKDPGLV RGFQKDPGLV KGFLKNTGLI KQFLKNTGLI RQFLKEPEFV RQFLKEPEFV REFLRNPSLV RWFQQNPGLV	TEAGHLGV-WTEAGHLDV-WTEAGHLDV-WFADALGV-SRIADQLQA-LTVABELGV-LDSANQLGE-RAFANELGV-RAFANELGV-RAFANELGV-	571	581	591 1
SEQ 3 SEQ 6 SEQ 10 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19 SEQ 22 SEQ 27 SEQ 30 SEQ 33		OILEEQD	VAEAKQTHDT	531	541	RGFOKDPGLA RGFOKDPGLA RGFOKDPGLA RGFLNTGLI RGFLKNTGLI RGFLKNTGLI RQFLKEPER RQFLKEPER RGFLREPER REFLRNPSLV	S61 WTFAQHLGV- WAWADELNV- FSTARKLGV- WTFAQHLDV- WTFAQHLDV- SRIADQLQA- ITVADELGV- ANLIINPEV- LRTAINLGU- LRTAINLGU- RAFANELGV-	571	581	591 1 QIRWGFTRRG QIRWGFSRRG CFGRAI QIRWGFTRRG QYKLALS QYKLALS QYKLALS QYKRAVERG QYHARVWERG QYDYAVKGHR
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SEQ 3 SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 14 SEQ 19 SEQ 27 SEQ 27 SEQ 30 SEQ 33 SEQ 35 SEQ 35 SEQ 42 SEQ 42 SEQ 42 SEQ 42 SEQ 42 SEQ 44	-NGKQAN -NGKQAN -NGKQAN -NGKQAN -NGKQAN -NGFELLN -ROIFKLD -ROIFKLD -TROGME -TADI-AB -TGHL-AB -TGHL-AB	GILEEQD	521 VAEAKQTHDT	1EVVSESHGG	541	S51 RGFQKDPGLA RGFQKNPGLV RQFLKEPEW RGFQKDPGLV RGFQKDPGLV RQFLKEPEFV RQFLKEPEFV REFLENESLV RWFQQNPGLV RWFQQNPGLV	S61 WIFAQHLGV- WAWADELNV- ESTARKLGV- WIFAQHLDV- WEFADKLGV- SRIADQLGA- LTVADELGV- ANLILNPEV- ANLILNPEV- LDSANQLGE- RAFANELGV- RAFANELGV- KDIJAGKVSS- WEWADDLNT-	571	581	591
SEQ 3 SEQ 6 SEQ 10 SEQ 12 SEQ 12 SEQ 14 SEQ 16 SEQ 19 SEQ 22 SEQ 27 SEQ 30 SEQ 35 SEQ 35 SEQ 35 SEQ 40 SEQ 42 SEQ 43 SEQ 43 SEQ 44 SEQ 42 SEQ 42 SEQ 42 SEQ 42 SEQ 42 SEQ 42 SEQ 42 SEQ 43 SEQ 44 SEQ	-NGKO-AN -NGKO-AN -SAHLANS ADEATAAEAM -NGKO-TROPELLN -TROCHE -DGRVTIOR -TROCHE -TADI-AR -DGLFTTAN -TGHL-AR -TGHL-AR -VGAM-VDA	GILEEQD	VAEAKQTHDT	TEVVSESHGG	541	REFERNESS REFORMEDIA REFORMEDIA REFUNESS REFUNESS REFUNESS REFUNESS REFUNESS REFUNESS REFUNESS REFUNESS REFUNESS REFUNESS REFUNESS REFUNESS REFUNESS REFUNESS REFUNESS REFUNESS REFUNESS REFUNESS REFUNESS REFUNESS REFUNESS	MTFAQHLGY- WAWADELNY- FSTARKLGY- WIFAQHLDY- WEFAQHLDY- WEFAQHLDY- MANILINE- LIVADELGY- ANLINE- LDSANQLGE- RAFANELGY- KDIJAGKVSS WSWADDLNT-	571	581	591
SEQ 3 SEQ 6 SEQ 10 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 16 SEQ 24 SEQ 24 SEQ 27 SEQ 30 SEQ 33 SEQ 35 SEQ 35 SEQ 42 SEQ 43 SEQ 44 SEQ 63 SEQ	-NGKQ-AN -SAHLANS ADEATAAEAM -NGKQ-AN -SAHLANS ADEATAAEAM -NGKQ-AN -NGFELKL E-DGRVTIOR -TROFELKL E-TADI-AR -TGHL-AR -TGHL-AR -UGAM-VDA -IGTL-AR	GILEEQD	521 VAEAKQTHDT	TEVVSESHGG	541	SS1 RGEQKDPGLA RGEQKNPGLV RGFLAEEMV RGFLKOPGLA RGFLKOPGLA RGFLKOPGLA RGFLKOPGLA RGFLKOPGLA RGFLKOPGLA RGFLKOPGLA RGFLKOPGLA RGFLKOPGLA RGFLKOPGLA RGFLKOPGLA RGFLKOPGLA RGFLKOPGLA RGFLKOPGLA RGFLKOPGLA RGFLKORMFGLV RLEGKNYGLV RLEGK	WTFAQHLGV-WAWADELNV-FSTARKLGV-WEFAQHLDV-WEFAQHLDV-WEFAQHLDV-WEFAQHLDV-WEFAQHLDV-WEFAQHLDV-Q-Q-Q-Q-Q-Q-Q-Q-Q-Q-Q-Q-Q-Q-Q-Q-Q-Q-Q	571	581	591
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SEQ 3 SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19 SEQ 27 SEQ 27 SEQ 30 SEQ 33 SEQ 35 SEQ 35 SEQ 42 SEQ 42 SEQ 42 SEQ 42 SEQ 42 SEQ 42 SEQ 42 SEQ 42 SEQ 42 SEQ 43 SEQ 43 SEQ 43 SEQ 43 SEQ 44 SEQ 42 SEQ 44 SEQ 42 SEQ 44 SEQ 44 SEQ 44 SEQ 45 SEQ 44 SEQ 45 SEQ 44 SEQ 45 SEQ 44 SEQ 45 SEQ 44 SEQ 45 SEQ 44 SEQ 45 SEQ 4	-NGKQAN -SAHLANS ADEATAAEAM -NGKQAN -SAHLANS ADEATAAEAM -NGPELLN -RDIFKLD E-DGRVTIOR -TROCHE -TADIAR -DGLFTTAN -TGHLRE -VGAM-VDA -IGTLRE -VGAM-VDA -IGTLRE	GILEEQD	VAEAKQTHDT	TEVVSESHGG	541	RGFQKDPGLA RGFQKNPGLV RQFLKEPBEV RGFLKEPBEV RGFLKEPBEV RQFLKEPBEV RQFLKEPBEV RQFLKEPBEV RQFLKEPBEV REFLKEPBEV REFLKEPBEV REFLKEPBEV REFLKEPBEV REFLKEPBEV REFLKEPBEV RKFQQNPGLV RLEQKNTGLV RLEQKNTGLV RLEQKNTGLV KLAEGSIGSG	S61 WIFAQHLGV- WAWADELNV- FSTARKLGV- WIFAQHLDV- WEFAQHLDV- WIFAQHLDV- LTVADELGV- ANLILINEV- LDSANQLGE- LDSANQLGE- RAFANELGV- KDIJAGKVSS WSWADDLNT- ECDAVILAR-	571	581	591
SEQ 3 SEQ 6 SEQ 10 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19 SEQ 22 SEQ 22 SEQ 24 SEQ 33 SEQ 35 SEQ 35 SEQ 40 SEQ 42 SEQ 44 SEQ 83 SEQ 83 SEQ 83 SEQ 83 SEQ 84 SEQ 84 SEQ 84 SEQ 84 SEQ 84 SEQ 85 SEQ 84 SEQ 84 SEQ 84 SEQ 85 SEQ 85 SEQ 85 SEQ 85 SEQ 85 SEQ 86 SEQ	-NGKQAN -NGKQAN -SAHLANS ADEATAREAM -NGKQMA -NGKQMA -NGKQMA -NGPELLN -POFFTIOR -TADIAR -DGLFTTAN -TGHLAE -VGAM-VDA -VGAM-VDA -VGMM-EG -FQLAEFPEPSQAE	GILEEQD	VAEAKQTHOT	TEVVSESHGG	541	RGFQKDPGLA RGFQKNPGLV RQFLEPEWR RGFQKNPGLV RQFLKEPEFV RQFLKEPEFV RQFLKEPEFV REFLKNPSLV REFLKNPSLV RFFLKNPSLV RFFLKNPSLV RKFQQNPGLV RKFQQNFGLV RKFQCNFGLV RKAGSEPDLR RAHLADPHNP	WTFAQHLGV- WTFAQHLGV- WTFAQHLDV- FSTARKLGV- WFFAQHLDV- WFFAQHLDV- WFFAQHLDV- WFFAQHLGV- LTVADELGV- LTVADELGV- LTVADELGV- RAFANELGV- RAFANELGV- WHARDLUT- ECDAVLAGE YFFAKELGV- QHARRLGV- QHARRLGV-	571	581	591
SEQ 3 SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19 SEQ 27 SEQ 27 SEQ 30 SEQ 35 SEQ 35 SEQ 35 SEQ 42 SEQ 44 SEQ 42 SEQ 44 SEQ 45 SEQ 44 SEQ 45 SEQ 4	-NGKO-AN -NGKO-AN -SAHLANS ADEATAAEAM -NGKO-TON -SAHLANS ADEATAAEAM -NGKO-TON -TROGRELIN -TROGRELIN -TROGRET -TADI-AR -DGLETTAN -TGHL-AE -VGAM-VDA -IGTL-AE -VGAM-VDA -IGTL-AE -VGAM-EG	GILEEQD	VAEAKQTHDT	1EVVSESHGG	541	GFQKDFGLA RGFQKDFGLA RGFQKNFGLV RQFLKEPEV RGFQKNFGLV RGFLKNFGLV RGFLKNFGLV RGFLKNFGLV RGFLKNFGLV RAPAINNFGLV RAPAINNFGLV RAPAINNFGLV RAPAINFGLV RELLERNFSW RPLERDFMW RPHLADPHMP	TEACHLOY- WIFACHLOY- ESTARKLGY- WIFACHLDY- WIFACHLDY- WIFACHLOY- LITVADELGY- RATAINIGY- LDSANQLGE- RAFANELGY- CDIAGKVSS WIWADDLNT- ECDAVILAR- YFARKELGY- QHARRELGY- QHARRELGY- QHARRELGY- QHARRELGY- QHARRELGY- LHEARKIGF-	571	581	591 1
SEQ 3 SEQ 6 SEQ 8 SEQ 10 SEQ 12 SEQ 14 SEQ 16 SEQ 19 SEQ 27 SEQ 30 SEQ 30 SEQ 33 SEQ 35 SEQ 40 SEQ 44 SEQ 42 SEQ 44 SEQ 65 SEQ 42 SEQ 44 SEQ 85 SEQ 8	-NGKO-AN -NGKO-AN -SAHLANS ADEATAAEAM -NGKO-TON -SAHLANS ADEATAAEAM -NGKO-TON -TROGRELIN -TROGRELIN -TROGRET -TADI-AR -DGLETTAN -TGHL-AE -VGAM-VDA -IGTL-AE -VGAM-VDA -IGTL-AE -VGAM-EG	GILEEQD	VAEAKQTHDT	1EVVSESHGG	541	GFQKDFGLA RGFQKDFGLA RGFQKNFGLV RQFLKEPEV RGFQKNFGLV RGFLKNFGLV RGFLKNFGLV RGFLKNFGLV RGFLKNFGLV RAPAINNFGLV RAPAINNFGLV RAPAINNFGLV RAPAINFGLV RELLERNFSW RPLERDFMW RPHLADPHMP	TEACHLOY- WIFACHLOY- ESTARKLGY- WIFACHLDY- WIFACHLDY- WIFACHLOY- LITVADELGY- RATAINIGY- LDSANQLGE- RAFANELGY- CDIAGKVSS WIWADDLNT- ECDAVILAR- YFARKELGY- QHARRELGY- QHARRELGY- QHARRELGY- QHARRELGY- QHARRELGY- LHEARKIGF-	571	581	591 1
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SEQ 27				
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SEQ 33	KKVNKSSL			
SEQ 35				
SEQ 38				
SEQ 40				
SEQ 42				
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SEQ 83	KIND DVT 107			
SEQ 85	HRVHVAKK			
Bacteria				
T44612	RYR			
NP 625402				
NP 295913				
AF320254	ETNLORAAAA	VAGK		
OYE family				
A£4875	YLDYPESAEY	MALHNERV		
A£4961	KCYVDYPPAT	ASS		
Ca2460	YNSYDESEKO	VIGKPLV		
Nc4452	VIDODESKEE	EKUYGBOB		
SCOYEL	VIDVDOVEED	T.KT.GWDKK		
ScOYE2	VIDVOTVEED	T.KT.GWDKN		
SCOYE3	YTDYPTYEEA	VDLGWNKN		
A36990	YNSYDESEKO	VIGKPLA		

Figure 1. A multiple alignment of the 2031 OR amino acid sequence from A. fumigatus (SEQ ID No3) along with related 2031 ORs from other fungi and bacteria (see Example 4) and OYEs. Regions 1-11, marked with * or #, refer to amino acids conserved between ORs but not OYEs.

Fungal 2031 ORs are given by the following SEQ ID No.: A. fumigatus, SEQ ID Nos. 3, 6 and 8; A. nidulans, SEQ ID No. 10; C. albicans SEQ ID Nos. 12 and 14; N. crassa, SEQ ID Nos. 16 and 19; M. grisea SEQ ID Nos. 22 and 44; S. pombe SEQ ID No. 24 (NP_595868); C. trifolii SEQ ID No. 27; F. sporotrichioides SEQ ID Nos. 30, 33 and 35; F. graminearum SEQ ID Nos. 38 and 83; M. graminicola SEQ ID Nos. 40 and 42; U. maydis SEQ ID No 85.

Bacterial ORs resembling 2031 are: T44612 (Pseudomonas putida), SEQ ID No. 86; NP_625402 (Streptomyces coelicolor), SEQ ID No. 87; NP_295913 (Deinococcus radiodurans), SEQ ID No. 88; AF320254 (Azoarcus evansii, SEQ ID No. 89.

Fungal ORs similar to the Old Yellow Enzyme family (originally identified in *S. cerevisiae*):

A. fumigatus, Af4875 and Af4961, SEQ ID Nos. 90 and 91 respectively; *C. albicans*, Ca2460 and A36990, SEQ ID Nos. 92 and 93 respectively; *N. crassa*, Nc4452, SEQ ID No. 94; *S. cerevisiae*, OYE1, OYE2 and OYE3, SEQ ID Nos. 95-97 respectively.

Details of the sequence searches that identified the ORs other than SEQ ID No. 3, and methods for the construction of multiple alignments are given in Example 4 hereinafter.

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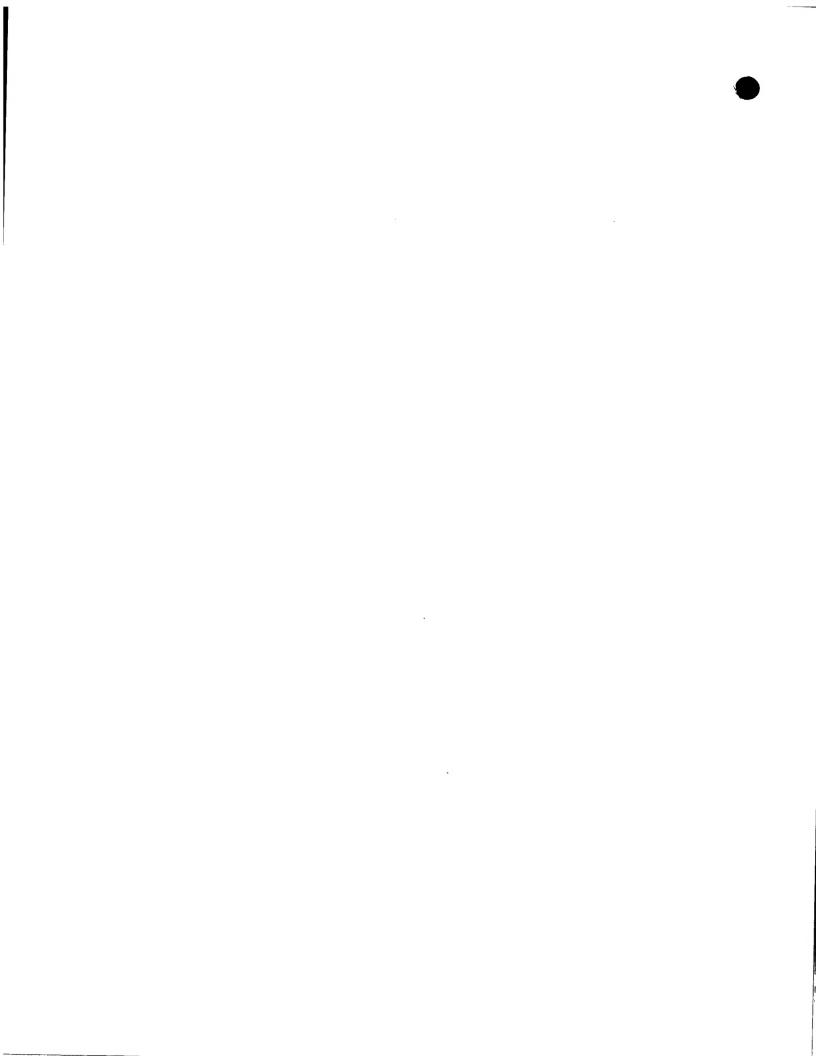
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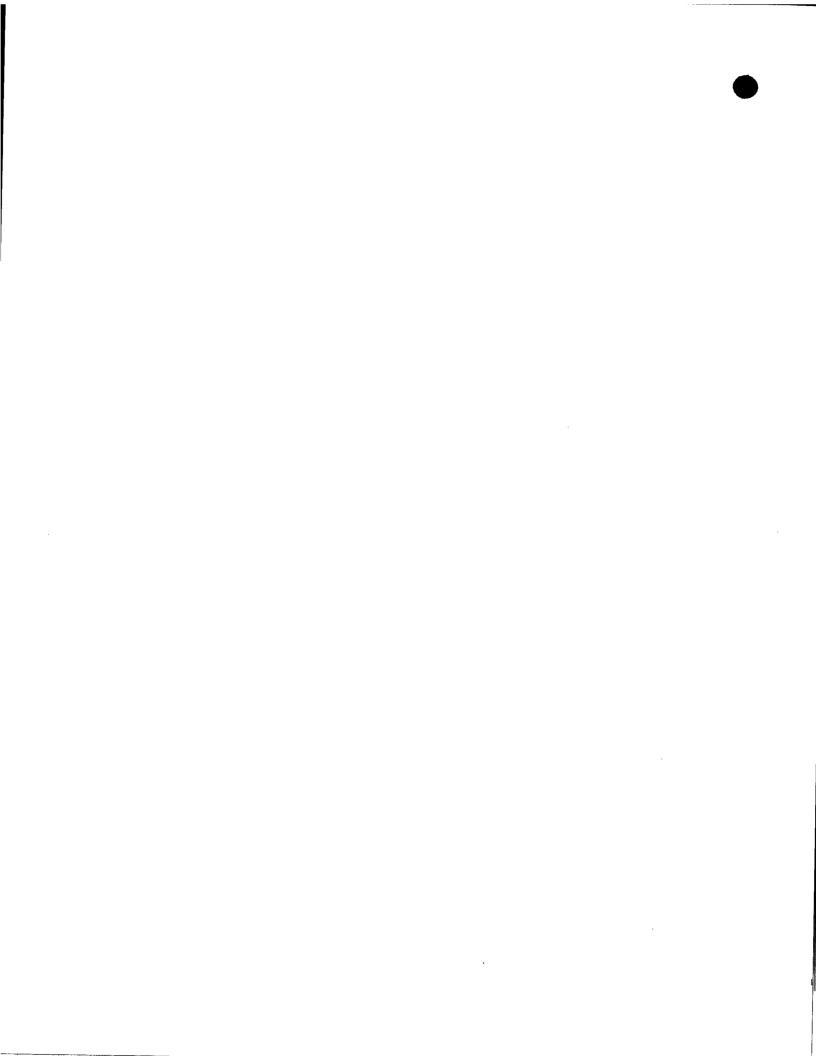
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SEQ 25 SEQ 26	GATTGCGCCC	GCTGAAG	C GCATCGTCG	A CITIMICON	C 1000101210		c cm>mc	CACCT	G TOGGRATGEG	G GTCGCAAGGC
SEQ 28	GATCGAGCC:	rctgaag	C GCATCACCA	C TTTCGCGCA	C MGICMGMGC	01010101011	C CMAMC	CACCT	G TOGGATGEG	G GTCGCAAGGC
SEQ 29	GATCGAGCC:	rctgaag	C GCATCACCA	C TITCGCGCA	C AGICAGAGO	0				
SEQ 32							C CMXMT	CAGAT	A GGTCATGCT	G GGAGAAAAGC
SEQ 34	GCTTGGACC'	rcrccgg	G ATATTGTGG	A CTTTGTACA	C AGCCAAAGC	W CAGAAGATT	G GTAT	TCAGCT	C TCGCACGCT	G GTCGTAAGGC G GTCGTAAGGC
SEQ 36	AATCGAGCC	TTTGAAG	C GCATCACTA	C TITIGCCCU	C AGCCAAAGC	- CAGAAGATI	G GTAT	TCAGCT	C TCGCACGCT	G GTCGTAAGGC G GCCGCAAAGC
SEQ 37	AATCGAGCC	U →TTGAAG	A GGTCGTC-G	A GTTTGCCCA	C TCCCAGAAC	- CAGAAGATO	A .TGATT	CAGTT	G GCGCATGCG	G GCCGCAAAGC
SEQ 39	GMICGAGCC									G GTCGCCAGGC
SEQ 41 SEQ 43	CTTCGACAT	G TTTTCCAAG	C TCGCCGCCG	C CGCCAAGGA	IG CACGGCAGC	- CIC-AICGI		CACAT	T GGCCATGCT	G GTCGCAAGGC
SEQ 82	TGTTGAGGG	ACTGCGA	A AGCACGTCG	A GTTTGCCC#	T GCCAACAAC	- TCTCTTATC	G GTATC	CAGAT	G CAACTGGCG	G GTCGCAAGGC C ATGCGGGAAG
SEQ 84	TCGGGATGC	ACACAAG	G CGCTGGTGT	C GGTGCTCAF	G TCCTTCACG	- GATGGTCTG	G GIGTA	-00001		C ATGCGGGAAG



	901	911	921	931	941	951	961	971	981	991
									**	****
SEQ 1	CACCACCGTT	GCGCCCTGGA	TCTCA				-TTCTCGGCC	ATCGCGACGG	AGAAGGTCGG	CGGATGGCCG
SEQ 2	CACCACCGTT	GCGCCCTGGA	TCTCA				-GCCAACGAT	ACCGCCTCCG	AGAAGATGGG	CGGCTGGCCA
SEQ 4	CAGCACCGTC	GCGCCATGGC	TCTCG				-GCCAACGAT -GCCAACGAT GTCGAGTCTG	ACCECCTCCG	AGAAGATGGG	CGGCTGGCCA
SEQ 5	CAGCACCGTC	GCGCCATGGC	TCTCG			ACCCCCCCAA	GTCGAGTCTG	AAGGCGGATG	AGAGCGTTGG	CGGGTGGCCC
SEQ 7	GAGTGCCGTT	GCGCCGTGGC	TGGCG			AA	CAAGGGCATC	GTCGCGACGG	AGAAGGTCGG	TGGCTGGCCG
SEQ 9	TTCGAACATC	GCCCCCTGGC	TCATG				CAAGGGCATC -TTGGAACAA	GTTGCAGATA	AATCTGTCAA	TGGGTTTGCC
SEQ 11	TTCTGGTCAG	CCCTTATTTT	TGCAC						ATACAACA	TGGTTGGCAA
SEQ 13	TGTTGAAGGG	GTACCATTCC	AACAA			CAGCGCGG	CAAGAGCGAG	CTTGCCGGCC	CCGAGCAGGG	TGGCTGGCCC
SEQ 15	CTCCACCAAG	GCCCCCTGGC	ACTAC		CTGT	GGGAGAAGGC	GGTGGCGCCC	TCGCCGGTGC	CGTTGGTGTT	GGGAGAGGCG
SEQ 17	TCCGATGGGC	GCGGGCACGC	GGGGA		CTGT	GGGAGAAGGC	GGTGGCGCCC	TCGCCGGTGC	CGTTGGTGTT	GGGAGAGGCG
SEQ 18	TCCGATGGGC	GCGGGCACGC	GGGGA	CACCCCCAGC	GGCGAGTATA	AGCCGAGAGA	GGGCTTACAG	GTCGTCGGAC	CCGAGTATGG	CGGCTGGCCT
SEQ 20	CAGCACAAAG	GCCCCCTGGC	ACGACICCII	CACCCCCAGC	GGCGAGTATA	AGCCGAGAGA	GGGCTTACAG	GTCGTCGGAC	CCGAGTATGG	CGGCTGGCCT
SEQ 21	CAGCACAAAG	GCCCCCTGGC	WCGWCICCII				TACACA	GTTGCGACTG	AAGCTCAAGG	TGGGTGGGAG
SEQ 23	TAGCACCACT	GCTCCTTAIC	CACC			GAGGCTCG	CGGCAAGGCG	CTGGCTCAGG	AGAGCGAGAA	CGGCTGGCCC
SEQ 25	TAGCACCCTG	CCACCGIGGA	TCACC			GAGGCTCG	CGGCAAGGCG	CTGGCTCAGG	AGAGCGAGAA	CGGCTGGCCC
SEQ 26	TAGCACCCTG	TOTAL CONTROL	TAAGC				-GTAAATGCT	GTCGCGGCGG	AAGAAGTGGG	TGGCTGGCCA
SEQ 28	CAGIIGCGIA	TCTCCCTGGC	TAAGC				-GTAAATGCT	GTCGCGGCGG	AAGAAGTGGG	TGGCTGGCCA
SEQ 29	CAGIIGCGIA						-GTAAATGCT			
SEQ 32 SEQ 34	CACCACACTC	GTACCGTGGC	TGGAC				-CGCAAGAAC	ACTGCTTTTA		mcccmcccca
SEQ 34 SEQ 36	TACTTCTCTA	TCTCCGTGGT	TGAGC				-ATCAACGCT	GTTGCCGCTA	AGGAAGTCGG	TGGCTGGCCA
SEQ 36 SEQ 37	TAGTIGIGIA	TCTCCGTGGT	TGAGC				-ATCAACGCT	GTTGCCGCTA	AGGAAGTCGG	TGGCTGGCCA
SEQ 37	CACCACTGTG	GCACCATGGT	TAAGC				-GGCGGCGAT	GTTGCTGGTG	AGGACGTCAA	ACCAMCCCCC
SEQ 41	GAGCACIGIO					GACT	GCCGAGTAAA	CGCGCCGGCA	AGGAGGCGGG	AGGAIGGCCG
SEQ 41										
SEQ 82	CTCCTGCGTT	GCTCCTTGGT	TAGAC	_~			-GCCGGACTT	GCCGCTGAAA	AGGCCGCTGG	TOGETEGECE
SEQ 84	GAAGGCCTCG	GACTGGTCAC	CTTTC		TACC	GCGGAGAAAA	GAAGCAAAAG	TTTGTGACGC	AGGAGGAAGG	1990199000
250 04		,						1071	1081	1091
	1001	1011		1031		1051		1071		
			-7				1061	******	****	
	*****	*****	*****				eccmmmccc	CAGCCCTTCG	CCAAGCCCAA	GGCCATGACG
SEQ 1	GACCCGCGTC	AAAGGGCCCG	GCGATATC				-CCCTTTGCG	CAGCCCTICG	CCAAGCCCAA	GGCCATGACG
SEQ 2	GAC-CGCGTC	AAAGGGCCCG	GCGATATC					CMMAACAACC	CTCTCCCGAA	GGAGATGACC
SEQ 4	GGC-CGCGTC	AAAGGCCCGA	CAAATGTG				-CCCTTTGCG -CCCTTCACC	CULLYAGAACC	CTGTGCCGAA	GGAGATGACC
SEQ 5	GGC-CGCGTC	AAAGGCCCGA	. CAAATGTG~-					CAMCCCMART	CCCTCCCCCC	GGCGCTGAGC
SEQ 7	GCG-GATGTG	GTGGGTCCGT	CGGGCGGG		GAGGAGC	AIMIGIATIO		CT CT COMMCC	CCACCCCAA	GGCCATGACC
SEQ 9	GAT-CGTGTG	ATCGGCCCGT	CCACCGTG				2001100110	CCM3 3 MMM3 C	CTCTTCCTAA	TGAGTTGACC
SEQ 11	GAC-AAAGCA	GTTGCTCCTT	CTGCATTG		GCATTC-		- AGACCAAAT CCATTTAGT	CARTCACACA	ATACACCACG	AGAATTGACT
SEQ 13	GAA-CATTGT	GTGGGGCCAT	CTACTGAG					CACA CCMMCC	CCTTCCCCAA	GGAGATGACC
SEQ 15	GAG-AACGTC	TGGGCCCCCA	GCGCCATC			AG	CTACAACGAG		GCACGCCGCG	GGAGCTGACG
SEO 17	TTT-GTGCCT	CGCTTGTTGT	CGAAAGTG					CTTTTCG	GCACGCCGCG	GGAGCTGACG GGAGCTGACG GGAGATGACC
SEQ 18	TTT-GTGCCT	CGCTTGTTGT	CGAAAGTG					GAGGACTTTC	CGAACCCCAA	GGAGATGACC
SEQ 20	GAT-GACGTC	TGGGCCCCGA	GCGCCATC					CACCACTTTC	CGAACCCCAA	GGAGATGACC
SEQ 21	GAT-GACGTC	TGGGCCCCGA	GCGCCATC				- AGGTGGGAC	GAAAACCACG	CTCAACCTCA	GGAGATGACC TAAGTTAACT
SEQ 23	AAT-GATGTT	TATGGACCAP	ATGAAGAC				71007000110	ANCONCEGG	CCACACCGCG	TGAGTTGACT
SEQ 25	GAC-GACGTT	GTGGCTCCCF	GCGCGATT				- CCTTMONG	ANCONCEGG	CCACACCGGG	TGAGTTGACT
SEQ 26	GAC-GACGTT	GTGGCTCCCF	GCGCGATT				* 7077077777	GCTGTGAACC	CAGTTCCCAA	GGCTTTCACG
SEQ 28	GAC-AATATC	GTTGCTCCC1	CGGCCATC			60	ACDAGAAAAT	GGTGTGAACC	CAGTTCCCAP	GGCTTTCACG
SEQ 29	GAC-AATATC	GTTGCTCCCI	CGGCCATC				, ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,			
SEQ 32							, ACAAGAAGCT			_
SEQ 34			·			G	ACAAGAAGC	GGCGTGAACC	CTGTTCCCAP	GGCCTTCACC GGCCTTCACC
SEQ 36	GAC-AACATT	GTTGCTCCT	CTGCCATC			GC	ACAAGAAGCI	GGCGTGAACC	CTGTTCCCA	GGCCTTCACC GGAGATGTCG
SEQ 37	GAC-AACATT	GTTGCTCCTT	CTGCCATC				-CCATCGAAC	CAGAAGCACG	CTGTCCCAA	GGAGATGTCG
5EQ 39	CAG-GATGTC	TGGGCGCCC	GTGCGATT		- MCCCATCAGI	CCTCCTCGAC	CGACCCTAG	GGAGGCTACT	ATGCGCCGAG	AGAGTTGTCG GCCCGCTACC
SEQ 41	GAG-GATGTT	GTGGGTCCGT	CGGGTGGGG	L GGACIIIAC	3 IGGGAIGAGA		TTTGG	TCAAAGTTTC	GCGTGCCCAG	GCCCGCTACC
SEQ 43							CCTTTTGCT	CCTGGCTACC	CTACCCCCC	TGCTATTACT AGCTCTCACG
SEQ 82	GAT-GACGTI	GTCGGACCTA	GCAACGAG				GCATATGC	CAAGGTCACG	TTACCCCTCC	AGCTCTCACG
SEQ 84	GAT-CGTGTC	GTCGCTCCT	r CGGCCATC							
			1121	1131	1141	1151	1161	1171	1181	1191
	1101	1111								
ane 1	CTCC TTCT	מיירכא פראפי	TCAAGAAGG	CTGGGTGGC	G GCCACGAAG	GCGCCATCG	C CGCCGG	GCGGACTTT	rcgagattc	A CAATGCGCAT A CAATGCGCAT
SEQ 1 SEQ 2	CIGGWIGY-C	ATCGAGCAG'	TCAAGAAGG	A CTGGGTGGC	G GCCACGAAG	GCGCCATCG	C CGCCGG	r GCGGACTTT	TOGAGATICA	A CAATGCGCAT A CAATGCGCAT
	ANGCAGGA-G	*ATCGAGGAT	TGAAGACCG	CTGGGTGGC	C GCTGTCAAA	C GGGCTGTTA	A GGCCGG	A GCCGACTTT	A TOGAGATOO	A CAATGCGCAT A CAATGCGCAT
SEQ 4	MUCUGGY-1	ATCGAGGAT	TGAAGACCG	CTGGGTGGC	C · GCTGTCAAA	C GGGCTGTTA	A GGCCGG	A GCCGACTTT	A TCGAGATCC	A CAATGCGCAT A TGGGGCGCAT
SEQ 5 SEQ 7	ACCCCCA-C	GTCCGTCAG	TGGTGGCGG	GTTTGCGAA	G AGCGCGCGG	TAGCGGTGC	A GGCTGG	GTGGATGTT	A TUGAGATUCA	A TGGGGCGCAT A CAATGCCCAC
SEQ 7	AAGGACGA-C	ATCGAGCAG'	TCAAGCGCG	A CTGGTTTGA	T GCGTGCAAG	GGGCCATTG	C CGCTGG	GUGGAUTTCA	TOURGATON	A CAATGCCCAC A TGGTGCTCAT
SEQ 11	AAAGATGA-A	ATCARACGT	TTGTTAAGG	A TTTTGGTGC	T GCTGCTAGA	A GAGCTGTTG	A AATCAGTGG	TTTGATGCAG	TIGAGATICA	A TGGTGCTCAT A TTGTGCTAAT
SEQ 13	GTTAATGA-	ATAAATTCA	A TTGTGGAAG	A CTTTGCCAA	T GCAGCTTGG	GGGCTGTGG	A AATCTCAAA	TTUGATGCC	TIGNMETER	A TTGTGCTAAT A CGCCGCCCAC
SEQ 15	GTCGAGCA-0	ATCCACGAG	C TCGTCGAGG	CTGGAAGGC	G TCTGCCCAG	GTGCCCTCA	A GGCCGG	TICGACCIC	TORGATION TO THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSPORT OF THE TRANSP	A CGCCGCCCAC A TGCGGCGCAT
SEQ 17	GTTGCGGA-0	ATCAAGGAT	A TCGTGCAAA	A GTTTGCGGT	G ACGGCGAGG	A TCACGGCCG	A GGCCGG	TICARIGGC	TOGUCATOR	A TGCGGCGCAT A TGCGGCGCAT
SEQ 18	GTTGCGGA-C	ATCAAGGAT	A TCGTGCAAA	A GTTTGCGGT	G ACGGCGAGG	A TCACGGCCG	A GGCCGG	S TICAMIGGO	N TOGRESATOR	A TGCGGCGCAT A CGGCGCTCAC
SEQ 20	GTTGAGGA-C	3 ATTGAGGGA	C TCGTCACCA	G CTTTGTGGA	C GCIGCCHAO			CTCCACATT	A TTGAGATTC	A CGGCGCTCAC
SEQ 21	GTTGAGGA-C	3 ATTGAGGGA	C TCGTCACCA	G CTTTGTGGA	C GCIGCCHIG			ም መጥጥር አጥርጥል	A TTGAAATTC	A TGGCGCTCAT
SEQ 23	GAAAAGCA-A	A TATGATGAA'	T TAGTGGATA.	A GITTGITGI	1 GCIGCGAAG		C A CCTCC	T TTTC ACCTC	A TTGAGATCC	A CGCCGCTCA-
SEQ 25	ACCGAGGRR	G TCGAGGGTC	r gggtgaaga	A GTTCGCCGA	G ICGGCCANG	. 0010111111	o no cmcc	m mmmcaccmc	A TTGAGATCC	A CGCCGCT
SEQ 26	ACCGAGGR-0	G TCGAGGGTC	T GGGTGAAGA	A GTTCGCCGA	G ICGGCCAMG.		N MCCTCC	T TTCGATGTT	A TCGAAATTC	A TGCAGCTCAT
SEQ 28	AAGGAGGA-	r atagagcaa	C TCAAGAGCG.	A CTACGIGGA	M GCGGCMINE	0 01100011100	2 MG CMCC	P PPCCAPCTT	A TOGARATTO	A TGCAGCTCAT
SEQ 29	AAGGAGGA-	r atagagcaa	C TCAAGAGCG.	A CIACGIGGA	M GCGCCARA					
SEQ 32										
SEQ 34								* **********	A TOGAGATOR	A TGCAGCTCAT
SEQ 36	AAGGAGGA-	I ATCGAGGAA	C TCAAGAATG	A CTTTCTGGC	T GCAGCMAAA	C GAGCCAWCC	C CCCTCC	T TTTGATGTC	A TCGAGATCC	A TGCAGCTCAT
SEQ 37	AAGGAGGA-	r atcgaggaa	C TCAAGAATG	A CTTTCTGGC	1 GCAGCIAAA	e ceasammer	A CCCTCC	› ጥጥጥGAጥGTT	A TTGAGATTC	A CAATGCTCAC
SEQ 39	TTGGATGA-	r atcgagget	T TCAAGAAGG	C GTTTGGAGA	de dedicard		7 7CCCCC	с стесатета	A TCGAAATCC	A CGGCGCGCAT
SEQ 41	GTCAGAGA-0	g atcaaggag	A TGGTCCAAG	A CTGGGGGAC	A GCAGCGAAA	n dddcddin.	A CCCCGG	T TTCGACGGT	A TCGAATTGC	A CGCCGCCCAC
SEQ 43	AAGGAGGA-'	I ATTAAGGCG	G TGATTGAGG	G TTTTGCCCA	C ACGGCCGAG		2 25 6266	א יייייכאכאכאכיי	A TOGACTICO	A TTTCGCTCAC
SEQ 82	CTTGAAGA-	G ATTGAACAG	T TGAAGGAGG	A CTTTGTTTC	C TOCCOLOR	T GEGCGTTG	A AGCTGG	G TATGACTAC	G TCGAACTTC	A CAGCGCTCAC
SEQ 84	ACCGAGGA-	C ATCAACAAG	T TGCAAGACA	A ATTCGTTCA	ADDADEST E	. 000001110				

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	1201	1211	1221	1231	1241	1251	1261	1271	1281	1291
ano 1										
SEQ 1 SEQ 2	GGATACCTGC	TGTCGTCATT	CCTCTCGCCG	GCCGCCAAC-						
SEQ 4	GGCTATCTTC	TGATGTCGTT	CCTCTCCCCT	GCGGTCAAC-						
SEQ 5	GGCTATCTTC	TGATGTCGTT	CCTCTCCCCT	GCGGTCAAC-						
SEQ 7 SEQ 9	GGGTATCTTC	TCTCGTCTTT	CCTATCACCG	TCTTCCAAC-						
SEQ 11,	GGTTATTTGA	TTAATGAGTT	CTATAGTCCT	ATTTCAAAC-						
SEQ 13	GGATGTTTAA	TACACCAATT	TTTAAGTAAA	TTGACAAAC-						
SEQ 15 SEQ 17	GGCTACCTCA	TGGCGCAGTT	CTTGAGCAAG	AAGACAAAC-						
SEQ 18	GGATACCTGT	TGGCGCAGTT	CTTGAGCAAG	AAGACAAAC-			ccccmcmcc	CCAMACTCCC	TOGGGTGTGA	CTTCTATTAA
SEQ 20	GGTTACCTGA	TCACCGAGTT	CCTTTCGCCG	CTATCAAACG	TAAGTGGAGA		GGGGCTGTGC			
SEQ 21 · SEQ 23	GGTTACCTGA	TATCGTCAAC	AGTTAGTCCT	GCCACTAAT-						
SEQ 25										
SEQ 26										
SEQ 28 SEQ 29	GGATATCTAC	TGCATCAATT	CTTGAGTCCG	GTAAGCAAT-						
SEQ 32										
SEQ 34				CTCACTAAC-						
SEQ 36 SEQ 37	GGATACKTGC	TTCACCAGTT	CTTGAGTCCA	GTCAGTAAC-						
SEQ 39	GGATACCTCC	TCCACGAATT	CATCTGCCTG	AGAGCAACA-				· · · · · · · · · · · · · · · · · · ·		
SEQ 41	GGGTACCTCA	TCCACGAATT	CCTCTCACCC	ATTACCAAC-		•				
SEQ 43	GGTTACCTGC	TGGCCCAATT	CCTGTCCGAA	GCCACCAAC-						
SEQ 82 SEQ 84	GGATACCTGA	TGCACTCGTT	CCTCAGCCCG	TTGACCAAT-						
		-				1251	1261	1371	1381	1391
	1301	1311								
								mememecaéa	TTCCCCACTT	GACTCGGGAC
SEQ 1			AACCGCAC	GGACCAGTAC	GGCGGGTCGT	TCGAGAACCG	CATCCGGCIG	TCTCTCGAGA	TTGCGCAGTT	GACTCGGGAC
SEQ 2			AACCGCAC	AGACCAGTAC	GGAGGCAGTT	TTGAGAATCG	CATCCGGCTC	AGTCTGGAGA	TCGCCAAGCT	CACCCGCGAA
SEQ 4 SEQ 5										
SEQ 7			AAGCGGAC	GGATGCGTAC	GGCGGGAGCT	TIGAGAACCG	CARCCCGCATC	TCTCTCGAAA	TCGCCCAGGT	CACCCGTGAC
SEQ 9			ACGCGCAC	CGACGAGTAC	GGTGGCAGTT	TTGAAAATAG	AACCAGATTT	TTAAAGGAAG	TTATCGATAG	TGTTAAATCA
SEQ 11 SEQ 13			AAGAGAGC	TGACCAATAC	GGGGGCTCAT	TTGAAAACAG	AGTTAGATTT	CTTTTACAAA	TAATTGAGAA	TATAAAACGA
SEQ 15			CAGCGTAC	CGACCAGTAC	GGTGGCTCCT	TCGAGAACCC	CCCCACCATT	CTTGGGGAGA	TTATTAAGGA	GTGCAGGAGG
SEQ 17			AGGCGCGG	GGATGAGTAT	GGCGGGTCGG	CIGAGAACAC	CCCCACCATT	GTTGGGGAGA	TTATTAAGGA	GTGCAGGAGG
SEQ 18	CAMMUMA TOTA	CCTGGCACGC	AGAAACGGAC	: AGACAAGTAC	GGCGGCAGCT	TTGAGAACCG	CACCCGGGTC	CTGATCGATA	TTATCAAGGC	CGTCCGGGCA
SEQ 20 SEQ 21	CATITIATI		AAACGGAC	AGACAAGTAC	GGCGGCAGCT	TTGAGAACCG	CACCCGGGTC	CTGATCGATA	TTATCAAGGC	CGTCCGGGCA
SEQ 23			GACCGCAF	TGACAAGTAT	GGTGGGACAT	TIGAGAAAC	INITITOTIA			
SEQ 25				. 						
SEQ 26 SEQ 28			CAAAGAAC	CGACGAGTAT	GG					
SEQ 29			CAAAGAAC	CGACGAGTAI	- GG======	macaca acc	• መአመሮክ ርክርጥ <u>ጥ</u>	CTCTTGGAAA	TCCTTGACCT	CATCCGCGCT
SEQ 32			AAU	CGACGAGIAI						
SEQ 34 SEQ 36										
SEQ 37			CAAAGAAG	CGATGAGTAL	GGTGGCAGCI	1 CGAGAACC	- CACHCCTCTC	ACAATGGAA	GTCGTCGACC	TTGTCCGCAG
SEQ 39			CCAGGACG	: GACAAGTACG	GGCGGTTCTI	TCGAAAACC	TACCCGTCTA	CTCATTGAA	A TCGTAACAGO	CGTCCGAGCC
SEQ 41 SEQ 43			CAGCGCAG	CGACGAGTAC	GGCGGCAGCC	TCGAAAACC	CATGCGGCT	ATCCTCGAG	TCACGGCCGA	GGTCCGCAGG TGCACGAGCT
SEQ 82			AAGCGTA	CGACAAGTAC	GGAGGTAGCT	TCGAGAACA	GCTCGATT	CTGCTCAAC	TTGCCCGTCG	TGCACGAGCT AATCCGCCAA
SEQ 84										
	1401	1411	1421	1431	1441	1451	1461	1471	1481	1491
										CCGGA
ano :	CCCCCCCCCCCC	CTCATGTGCC	: C		GTTT	CCTGCGCAT	TCGGCCTCGC	ACTGGTGCG	A GGAGACCCT	CCGGA
SEQ 1 SEQ 2	GCCGTCGGCC	CTCATGTGCC	: C	-,	GTTT	CCTGCGCAT	TCGGCCTCGC	ACTGGTGCG	A GGAGACCCTC	CCGGA
SEQ 4	AATGTGCCCA	. AGGATATGCC	T		GTCT7	CCIGCGGGI	- maacccaacc	ATTCCCTCC	GGAGGTGCAC	CCGAA
SEQ 5	AATGTGCCCA	AGGATATGCC	T		CTGT	TCTGCGTAT	C AGCGCCACG	AGTGGTTGG.	A GGGTCAGCC	GTGGC
SEQ 7 SEQ 9	GTGATTCCCG	CCAACGTTCC	T		GTTT	TCTCCGTGT	C TCCGCGACG	ACTGGATCG	A GGAGACCCT	CCCGA
SEQ 11	AGTATTCCAA	ACGATGTTCC	: A				m ccnnmcmch	с атааттста	G TGATCCG	
SEQ 13	AAGATAGAAA	CACC	: G		ATTT	CITAMAGII	c mcccccacc	G AGTGGATGG	A GTACACC	
SEQ 15 SEQ 17	CACCTCACTC	AGGACATGCC	TGAAGAGGA	G GCGAAGAAG'	r TTGTGGTGG	AATCAAGCT	G AACAGTGCG	G ATTGGCAGG	C GGGACGCGA	r ggaA r ggaaag
SEQ 18	CAGGTGACTG	AGGCGGTGGG	TGAAGAGGA	G GCGAAGAAG	r Treredies	AUTOMICO T	C MCCCCCBACCI	C AATGGATGG	A GTACGCCGG	C
SEQ 20	GTGATTCCCG	AGGAGATGC	: A			COTOCOLLIA	c mcccccacc	C ANTEGNTES	A GTACGCCGG	c
SEQ 21	GTGATTCCCG	AGGAGATGCC	. A		TTGT	TTATAGAGT	A ACGGCTACA	G ATTGGTTGC	C CAAAGGACA	A
SEQ 23 SEQ 25										
SEQ 26										
SEQ 28										T CAATTCAAAG
SEQ 29 SEQ 32	GCCATCCCCG	AAACTACACO	T		GYCC	r cerréerer	C MGIGGIAIGE			
SEQ 34										
SEQ 36										
SEQ 37 SEQ 39	CATT				<u> </u>					C ATCGGC
SEQ 41	GCGATGCCCI	CCAGCATGC	: T		CTC1		m andaccerc	G AGTTCCAGG	A GAAG	
SEQ 43	CGGACGAGC	AGAATTTCA:	r c			2 CVICANTII	C ACTCGAACT	G ACTGGCTGG	A GAACAACCC	T GAG
SEQ 82	GTTATGCCTC	AGGACATGCO A ACAAGGGT-	,		CTCT	G GGTGCGCGT	C AGCTCCACC	G ACTGGGCCG	A CCAAGCGCA	C CAA
SEQ 84	0.1.1.10000									



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		1501								9	****
SEQ 1		-	GCAGAGCTGG	AAGTCGGAGG	ATACCGTGCG	GTTCGCGCAG	GAGCTGGTCA	AGCAGGGCGC AGCAGGGCGC	CGTTGATCTG	ATCGATATCA	GCAGCGGTGG
SEQ 2			GCAGAGCTGG	AAGTCGGAGG	ATACCGTGCG	ATTUCCECAG	ATCCTGGCAG	AAACGGGTTA	CGTTGACGTG	CTTGACGTGA	GCAGTGGCGG
SEQ 4		CAA	GCCCAGCTGG	CCACGCGTGG	ACACTGTCCG	ATTTGCGAAG	ATCCTGGCAG	AAACGGGTTA	CGTTGACGTG	CTTGACGTGA	GCAGTGGCGG
SEQ 5 SEQ 7		CAA	GCCCAGCIGG	GATATGC	AGAGCTCGCT	GGAGCTGGTC	AAGAAGCTGC	CCGAATGGGG	CATTGACCTG	GTGGATGTCA	GCTCCGCCGC
SEQ 7			GGAATCGTGG	AAGCTCTCTG	ACTCCGTCCG	CTTCGCCGAA	GCCCTCGCTG	CCCAGGGCGC	TATTGACCTG	ATCGACGTCT	CATCTEGTEG
SEO 11			-GAAGCTTGG	ACTATTGAAG	ATTCCAAAA-	AATTAGCT	GACATTTAG	TAGAAAAGGG	TATTGCTTTG	ATCGACGTTA	CATCAGGTGG
SEQ 13			-GAAGCGTGG	TCTACGGAAG	ATGCATTGA-	AGTTGGCC	GAICIIGIIA	CCCACCTCCC	CCTCCACCTC	CTCGACGTCT	CTTCCGGCGG
SEQ 15		GGCCA	GCCCTCGTGG	GACCTCCAGC	AGACCATTG-	AGCTCGCC	CACCERTTE	ACCACTCGGG	GATCGACTTT	GTCGAGGTTA	GCGGTGGCAG
SEQ 17		AGGAGGAGGA	GGAGACGGAT	ACGGCGGAGG	AGGTGTTGA-	AGCAGATT	GAGCTTTTTG	AGCAGTGGGG	GATCGACTTT	GTCGAGGTTA	GCGGTGGCAG
SEQ 18		GAGGAGGA	GGAGACGGAT	ACGGCGGAGG	AGGIGITON	AGCTTGCC	AAGCTCCTCC	CGGACCTGGG	TGTCGACCTG	CTCGACGTCA	GCTCGGGCGG
SEQ 20 SEQ 21		GA	CCCTACCTGG	GACCTCGAGC	AGAGCACAC-	AGCTTGCC	AAGCTCCTCC	CGGACCTGGG	TGTCGACCTG	CTCGACGTCA	GCTCGGGCGG
SEQ 21		GA	GGATGG	GAGATAGAAG	ATACAGTTG-	CATTAGCA	GCGAGGCTTC	GCGATGGTGG	TGTTGACTTG	ATAGATGTTA	GCTCTGGTGG
SEQ 25								GCGATGGTGG			
SEQ 26											
SEQ 28											
SEQ 29			mca a a comec	NCNCECCNGC	AGACTTG	TCAACTCGCG	CGTATCTTGC	CCAAGCATGG	AGTAGACTTG	GTGGACGTCA	GCTCAGGCGG
SEQ 32		ACGAGTTTCC	TGAAAGCTGG	ACAGICGAGC	AGACTI			CCAAGCATGG			
SEQ 34 SEQ 36											
SEQ 37											
SEQ 39	3						22222222	CCGACTTGGG	CGTTGATCTC	CTCGACGTGT	CTTCCGGTGG
SEQ 41		aagaagtt	CGGAAGCTGG	GATGTCGAAA	GCACGATCA-	AGATOTCC	GAGGCCCTCG	AGGCCGCGG	CATGGATTTT	GTCGAGACGA	GCGGCGGCAC
SEQ 43	,		-GGTTTCAAG	ACTOTOTO AGG	AGGCGGIGC-	AGCTTGCA	CACCAGTTAG	CAGACCGTGG	TGTCGATGTT	TTGGATGTTT	CCAGTGGTGG
SEQ B2	:	TACGAGGG	CCACTCTTCG	ACCOTTGAGC	AGACGGTTG-	AACTCGCC	AAGATGCTCC	AAGAGGCTCG	AGTCGACCTG	CTAGACGTCA	GCTCCGGCGG
SEQ 84	•		Cancitation							1681	
		1601	1611	1621		1641		1661			
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SEQ 1		TGTTCTCGCG	CAG								
SEQ 2 SEQ 4		CACTCATTCG	GAG								
SEQ 5		CACTCATTCG	GAG								
SEQ 7		GAACCACAAG	GAC								
SEQ 9		TGTCCACGCC	GCG								
SEQ 11	L	TAACGATTAT	AGA								
SEQ 13											
SEQ 15	,	CAACAACAAG	CCTCAGGTAA	GTTTTGGTGT	TGTTTGAGGG	ATGGGGCAAG	GGGTTGTCTG	TCGTGAACAA	CAAAAGGGGC	ACGGAACAAA	TGCTAACGCC
SEQ 17	2	TTATGAGGAT	CCTCAG								
SEQ 20		AAACTCGGTG	GCC								
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SEQ 23		TAATCACAAG	GAT								
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SEQ 4:		CTATGAGAGT	TTT								
SEQ 8		CATCCACAAG	ATG								
SEQ 8		CCTGGTTCCA	TTC								
				1701	1731	1741	1751	1761	1771	1781	1791
		1701	-10								
		####	##########	#########	#########	##			TOGGCCCTCA	GARGGCCGTC	GGCGAC
SEQ 1				CAG	AAGATCAAGI	CCGGCCCIGC	CHICCHGOI	COTTTTCCC	TESCCETGA	GAAGGCCGTC	GGCGAC
SEQ 2				CAG	AAGATCAAGT	CCGGCCCTGC	CITCCAGGI	CCCTTTGCCT	TTGCCGTCA	GAACGCCGTC	GGGGAC
SEQ 4				CAC	CATATCCACC	CGAAGCCAG	CTTCCAGGC	A CCCTTTGCT	A TTGCCGTCA	GAACGCCGTC	GGGGAC
SEQ 5				CAG	AAGATCAACO	TGCACACGG	CTACCAGAC	GACCTGGCC	GGCAGATTC	CCAGGCCATC	CGAGCG
SEQ 7 SEQ 9				CAG	AAGATCAAGT	CCGGGCCGG	TTTCCAGGC	r cccttcgct	TGGCTATCA	A GAAGGCCGT	GGCGAT
SEQ 1											
SEQ 1		T	GCAAATCTAG	ATATCTATT?	AATGACGACA	AACAACTAC	CONCORRECT	COCTIGGON	AGCAGATCC	CGCGGCCGT	CACGAGGCCG
SEQ 1	5			CAG	AAGATCAACC	TCCACACCT	COCCCAGAI	- TTCTTCCTC	AGTTCGCCA	GATCATCCG	C ACCAAGT
SEQ 1		ATACAGATGG	CCAACGGTCC	CAAGCCCGAF	AMGICCGAAC	GCACCATGG	CCGCGAGGC	TTCTTCCTC	AGTTCGCCA	A GATCATCCG	C ACCAAGT C GGCGAT
SEQ 1		ATGG	COMMUGISTED	CAI	AAGATCGAGG	TCACGCCGT	CTACCAGAT	C GACCTGGCA	CCAAGATCC	CGAGGCCGT	GGCGAT
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SEQ 1 SEQ 2	AAGCT	GCTGGTTGCC	GCCGTGGGTG	CCATCACC					AACG	GCAAGCAGGC
SEQ 4	AAACT	CGCAGTGGCA	TCAGTGGGTA	TGATTGCC					AGCG	CGCATTTGGC
SEQ 5	AAACT	CGCAGTGGCA	TCAGTGGGTA	TGATTGCC			002 00002 CM	ACTUACACCCA	CCCCACCACC	CGACTGCAGC
SEQ 7	GCTGG	CGCGTCGACT	CTTGTGGGTG	CCATCACG:	GAICACCGAI			AGIICAGGA	AACG	GTAAGCAGGC
SEQ 9	AAGCT	ATTIGUE	TECETTEETE	GGCTTGAA					A	AAGATCCTGA
SEQ 11 SEQ 13	CGATG	TTTGATCGCA	TGCAGTGGAG	GATTAGAT				CGTCCAGGAG	AACCAGGATG	GAGACATATI
SEQ 15	GCAAGCAGCT	CCTCGTCGGT	GCCGTCGGCT	19910100					ACTC	GTCAGGGCAT
SEQ 17	TCCCCAAGCT	TCCTCTCATG	GTCACCGGCG	GCTTCCGC				·	ACTC	GTCAGGGCAT
SEQ 18 SEQ 20	TCCCCAAGCT	CCTCTCATG	GCGGTCGGCA	ACATCAAC					ACGG	CTGACATTGC
SEQ 20 SEQ 21	AGGTT	GCTCATAGGC	GCGGTCGGCA	ACATCAAC					ACGG	CTGACATTGC CTCTTACGGC
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SEQ 32	AGTGT	ACTTGTTTCA	GCAGTAGGTG	GAATCAAG						
SEQ 34										
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SEQ 39 SEQ 41										CCATGGTCGA
SEQ 43	ATGGT	GGTCTACACC	ACCGGCGGCT	TCAAGACG					ATAG	GTACCCTTGC
SEQ 82	AAGAT	GTTGATCAGC	ACTGTTGGTA	GCATCAAG					CCGTGG	GAATGATGGA
SEQ 84	ATCGAACC	CGACGCGTCC	AAACGCATGC	TCGTCGGGG-						
	1901	1911	1921	1931	1941	1951	1961	1971	1981	T33T
SEQ 1	GAATCAG	ATTCTAG	AGGAGCAG							
SEQ 2	GAATCAG	TTGTTGG	AGAAGGAC							
SEQ 5	C 3 3 TTC C	TTGTTGG	AGAAGGAC							
SEQ 7	CCACCCAATG	CTGTCGGGAC	CTGAACCC							
SEQ 9	CARCARG	CTGCTTG	AGGAGGAG							
SEQ 11	ATTGCTCAAC	CACTTURATE	CTAATGGT							
SEQ 13 SEQ 15	CATCCAGCGC	GAGAACGGCG	CCAAGACT							
SEQ 17	GGAGGCC	GCTTTGG	AATCCGAT							
SEQ 18	GGAGGCC	GCTTTGG	AATCCGAT					CRCCTCACCC	AATCACATGG	CGGCAAGACC
SEQ 20	GCGCGATGTC	GTGGATGAGC	AGGGCGCCGA	GAAGGTGGCC	GAGGCCAAGC	AGACGCATG	CACCAMCCA	CTCCTCAGCG	AATCACATGG	CGGCAAGACC
SEQ 21	GCGCGATGTC	GTGGATGAGC	AGGGCGCCGA	. GAAGGIGGCC						
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	2001	2011	2021	2031	2041 .					
SEQ 1	GATATCGACO	TTGCGCTGG	TGGCCGTGGG	TTCCAGAAG	ATCCCGGTC	r GGCCTGGAC	G TTTGCTCAG	C ACCICGGCG	r c	
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SEQ 4	GGACTGGACC	: TTGTGCTGG	r TGGACGTGGC	, licendand	1 ACCCGGGGG		c mccccccac	C ACCTCAATG	r A	
SEQ 5	GGACTGGACC	: TTGTGCTGG	I IGGACGIGG	. IICCAGAAG	1 ACCCCCCCC		- 1000000707	N NORTHCECCS	r G	
SEQ 7 SEQ 9	GGATTGGAT	TTGCGCTTG	GGGACGTGGT	TTCCAGAAG	ATCCCGGTC	T GGCGTGGAC	T TTCGCGCAG	C ATCTTGATG	r T	
SEQ 11	ACATTTGATO	TTGCTTTGA:	r CGGTAGAGG	TITITIA	1 Miconday		m nmmccmcac	C DATTCCARG	C A	
SEQ 13	GACTTTGAT	TAGCATTGA:	r aggtaaagg <i>l</i>	A TTTCTCAAA	A ACACTGGAT	T GATCAGCCG	m cmcccccyc	C ACTTEGETS	т т	
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SEQ 17	GATTGCGACA	TGATCGGTA	r CGGACGCCC	GCCATCATC	A ACCCTTCGC	T TCCCGCCAA	C TTGATCCTC	A ACCCGGAGG	T G	
SEQ 18 SEQ 20	AAGGCGGAT	TGGTCCTCA:	r TGCTCGCCAC	3 TICCIGCGC	J AGCCIGAGI		C ACCCCCCAM	A ACCUTEGGG	т с	
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SEQ 23	GAT	TTACTTTTG'	r cgcaaggga	3 TTCTTAAGG	W WCCCGICGI	1 0010011101				
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SEQ 41							C AMCAMCGCG	C CCAAGGTGT	C CAGCATTAT	C AAATACGCCA
SEQ 43		 ATAGGCA 	T CGGGCGCGC	A GCCGGTTCG	G AGCCGGACC	T CGCCAAGGA	C MICHICAL	C ATCTGAACA	C T	
SEQ 82	CCCTTGGAT	TTGTGGCTT	C AGGCCGTCT	G GCCCAGCAG	T CGATTCAGA	G CGGAGAGT	T GATGCGGT	C TGTTGGCAC	G T	GGATTGA
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SEQ				GAGA	TCTCCATGGC	TAATCAGATC	CGAIGGGGII	TCTCGCGGCG	CGGTGCTGGT	CCTTACCTCA	GGAAGAAACT
SEQ				GAGA	TCTCCATGGC	GGTGCAGTTT	GGCAGGGCCA	TTTAG			
SEQ											
SEQ				AGAC	TCCACCAGGC	CTTGCAGTTA	GGTTGGGGTT	TCTGGCCCAA	CAAACAACAA	ATTGTTGATT	TGATTGAAAG
SEQ				CAAT	TCAGAACAGC	ACCTCAATAT	AAGIIGGCCI	CTCTTACCAG	CAGGCCCAAG	AAGTTGACCA	CTGTTCCTTA
SEQ				GATG	ATCCGGATCC	CCCCTTGTTC	GACAAGAAGA	GGGCTGAGCC	GCACTGGATC	GTTGAGAAGT	TGGGCATGAA
SEQ											
SEQ				AATG	TGCAGTGGCC	TCACCAATAC	CACAGAGCAG	TGTGGCGCAA	GGGTGCAAGG	ATTTGA	
SEQ				AATG	TGCAGTGGCC	TCACCAATAC	CACAGAGCAG	TTARGEGREACA	CAGAAAGTTA	CGTTGA	
SEQ	23			AATG	TTGCATGGCC	AGTTCAGIAI	GACIAIGGAG				
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SEQ									TCCADACAAA	GTGAACAAGA	GTTCTTTATA
SEQ				GAGG	TCAAGATGGC	GAACCAGATT	GATTGGAGCT	TCAAGGGACG			
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SEQ	41							CCAAGGGCGA			
SEQ		TGGGGGAGGA	CGAGTTTGTG	CTGCAGTTGA	TCCAGATCGC	TCATCAGATC	GCATGGGGTT	TCGGTGGCAG	AGCTAAGAAG	AACGCTCCCA	AGCTTGTCTT
SEQ SEQ		TGTCCTACCC	AAGCTGGACC	GAGGATGCTA	GTGTAGCGCT	GATGGGTACC	AGGGCAGCTG	GCAACCCGCA	GTACCATCGC	GTTCACGTGG	CTAAGAAGTG
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		2201	2211	2221	2231		2251			~	
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SEQ		GTACAAGCAG	TCTATTTTCG	ATGTATAG							
SEQ		CGAGAAGATA	TAA								
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SEQ		GTCCATTGTT	GGTGCTGGTG	TTGAGGTGGT	ACGICACGIT	CCAACCCCAI					
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SEQ SEQ	36 37 39 41 43 82 84	A	2311	2321	2331	2341	ATCT	2361	gaggtgggg	GGGTGACGC	A GTTGATGGCG
SEQ SEQ	36 37 39 41 43 82 84	A	2311 GAGTTGAAG/	2321 TGATACCTCA	2331 A TAGACGATCA A TAGACGATCA	2341 AATGGACCCTT	2351	2361	2371	2381	A GTTGATGGCG 2391 C ACAGTAGCTG
SEQ SEQ SEQ	36 37 39 41 43 82 84 84	A	2311 GAGTTGAAG/	2321 TGATACCTCA	2331 A TAGACGATCA A TAGACGATCA	2341 AATGGACCCTT	2351	2361	2371	2381	A GTTGATGGCG 2391 C ACAGTAGCTG
SEQ SEQ SEQ SEQ SEQ SEQ	36 37 39 41 43 82 84 11 22 34 45	A	2311 A GAGTTGAAGA	2321 TGATACCTCZ	2331 TAGACGATCA	2341 A ATGGACCCT'	2351 r GCATATTAT	2361	2371 TGCGTATGT	GGGTGACGC	A GTTGATGGCG
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SEQ SEQ SEQ SEQ SEQ SEQ SEQ SEQ	36 37 39 41 43 82 84 82 84	A	2311 GAGTTGAAG	2321 TGATACCTC/	2331 TAGACGATCI	2341 A ATGGACCCTT	2351 GCATATTAT	2361	2371 TGCGTATGT	2381 CAAGGTATT	A GTTGATGGCG 2391 C ACAGTAGCTG
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SEQ SEQ SEQ SEQ SEQ SEQ SEQ SEQ SEQ SEQ	36 37 39 41 43 82 82 84 11 22 4 4 25 7 7 19 21 13 21 21 21 21 21 21 21 21 21 21 21 21 21	A	2311 GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA GAGTGAAGA GAGTTGAAGA GAGTGAAGA GAGTTGAAGA GAGTGAAGA GAGTTGAAGA GAGTGAAGA GAGTTGAAGA GAGTTG	2321 A TGATACCTCA TGATACCTCA CAAGAAGCTC	2331 A TAGACGATCA TAGACGATCA GCCAAGTTT	2341 A ATGGACCCTT ATGGACCCTT	2351 GCATATTAT	2361 TCTCGTCTCC	2371 TGCGTATGT	2381 CAAGGTATT	2391 C ACAGTAGCTG
SEQ SEQ SEQ SEQ SEQ SEQ SEQ SEQ SEQ SEQ	36 37 39 41 43 82 84 11 22 4 4 5 7 19 11 11 12 11 11 11 11 11 11 11 11 11 11	A	2311 GAGTTGAAGA GAGTTGAAGA TGAGCGAGCS TGAGCGAGCS TGAGCGAGCS	2321 TGATACCTCZ TCAAGAAGCTC	2331 A TAGACGATCI A TAGACGATCI G GCCAAGTTT	2341 ATGGACCCTI	2351 GCATATTAT' GCATATTAT'	2361 TCTCGTCTCC	2371 TECGTATGT	2381 CAAGGTATT	A GTTGATGGCG 2391 C ACAGTAGCTG
SEQ SEQ SEQ SEQ SEQ SEQ SEQ SEQ SEQ SEQ	36 37 39 441 43 82 884 10 12 14 15 17 17 17 18 11 11 11 11 11 11 11 11 11 11 11 11	A	2311 GAGTTGAAGA GAGTTGAAGA GAGTTGAAGA TGAGCGAGCC TGAGCGAGCC	2321 A TGATACCTC2 TGATACCTC2 TCAAGAAGCTC CAAGAAGCTC	2331 A TAGACGATC: A TAGACGATC: GCCAAGTTTT GCCAAGTTTT	2341 ATGGACCCTT ATGGACCCTT TAG	2351 GCATATTAT	2361 P TCTCGTCTCC	2371 TGCGTATGT	2381 CAAGGTATT	A GTTGATGGCG 2391 C ACAGTAGCTG
SEQ SEQ SEQ SEQ SEQ SEQ SEQ SEQ SEQ SEQ	36 37 37 41 43 82 84 11 22 24 25 27 21 21 21 21 21 21 21 21 21 21 21 21 21	A	2311 AGAGTTGAAGA GAGTTGAAGA GAGTTGAAGA TGAGCGAGC TGAGCGAGC	2321 TGATACCTCI TGATACCTCI CAAGAACCTC CAAGAACCTC	2331 A TAGACGATCI A TAGACGATCI GCCAAGTTTT	2341 A ATGGACCCTI A ATGGACCCTI T AG T AG	2351 GCATATTAT	2361 TOTOGTOTO	2371 TGCGTATGT	2381 CAAGGTATT	2391 A CACAGTAGCTG
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SEQ SEQ SEQ SEQ SEQ SEQ SEQ SEQ SEQ SEQ	36 37 37 37 41 41 41 42 42 43 44 45 47 47 47 47 47 47 47 47 47 47 47 47 47	A	2311 A GAGTTGAAGA GAGTTGAAGA TGAGCGAGC TGAGCGAGC	2321 TGATACCTCA TGATACCTCA TGATACCTCA CAAGAACCTC CAAGAACCTC	2331 A TAGACGATCI A TAGACGATCI GCCAAGTTTT GCCAAGTTTT	2341 A ATGGACCCTI A ATGGACCCTI T AG	2351 GCATATTAT	2361 TOTOGTOTO	2371 TGCGTATGT	2381 CAAGGTATT	2391 A CAGTAGCTG ACAGTAGCTG
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	0511	2521				2561	2571	2581	2591
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AATATAAAA	A GCGGGGAATG	GCTTGACCCC	2531 GCGCAGAATG	2541 TCGATCTCTT	2551 CGCAAACTCT	2561 	2571 GACGCTCAGC	2581 	- G -
AATATAAAA	A GCGGGGAATG	GCTTGACCCC	2531 GCGCAGAATG	2541 TCGATCTCTT	2551 CGCAAACTCT	2561 	2571 GACGCTCAGC	2581 	- G -
AATATAAAA	A GCGGGGAATG	GCTTGACCCC	2531 GCGCAGAATG	2541 TCGATCTCTT	2551 CGCAAACTCT	2561 CGGTGTATAG	2571 GACGCTCAGC	2581 	- G - -
AATATAAAA	A GCGGGGAATG	GCTTGACCCC	2531 GCGCAGAATG	2541 TCGATCTCTT	2551 CGCAAACTCT	2561 CGGTGTATAG	2571 GACGCTCAGC	2581 AACGATCAAG	- G -
AATATAAAA	A GCGGGGAATG	GCTTGACCCC	2531 GCGCAGAATG	2541 TCGATCTCTT	2551 CGCAAACTCT	2561 	2571 GACGCTCAGC	2581 AACGATCAAG	- G - -
AATATAAAA	A GCGGGGAATG	GCTTGACCCC	2531 GCGCAGAATG	2541 TCGATCTCTT	2551 CGCAAACTCT	2561 CGGTGTATAG	2571 GACGCTCAGC	2581 ————————————————————————————————————	- G - - -
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AATATAAAA	A GCGGGGAATG	GCTTGACCCC	2531 GCGCAGAATG	2541 TCGATCTCTT	2551 CGCAAACTCT	2561 CGGTGTATAG	2571 GACGCTCAGC	2581 ————————————————————————————————————	- G
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AATATAAAA	A GCGGGGAATG	GCTTGACCCC	2531 GCGCAGAATG	2541 TCGATCTCTT	2551 CGCAPACTCT	2561 CGGTGTATAG	2571 GACGCTCAGC	AACGATCAAG	
AATATAAAA	A GCGGGGAATG	GCTTGACCCC	GCGCAGAATG	2541 TCGATCTCTT	2551 CGCAAACTCT	2561 CGGTGTATAG	2571 GACGCTCAGC	AACGATCAAG	- G
AATATAAAA	A GCGGGGAATG	GCTTGACCCC	2531 GCGCAGAATG	2541 TCGATCTCTT	2551 ———————————————————————————————————	2561 CGGTGTATAG	2571 GACGCTCAGC	AACGATCAAG	- G
AATATAAAA	A GCGGGGAATG	GCTTGACCCC	GCGCAGAATG	2541 TCGATCTCTT	2551 CGCAAACTCT	2561 CGGTGTATAG	2571 GACGCTCAGC	AACGATCAAG	- G
AATATAAAA	A GCGGGGAATG	GCTTGACCCC	GCGCAGAATG	2541 TCGATCTCTT	2551 CGCAAACTCT	2561 CGGTGTATAG	2571 GACGCTCAGC	2581 AACGATCAAG	- G
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AATATAAAA	A GCGGGGAATG	GCTTGACCCC	GCGCAGAATG	2541 TCGATCTCTT	2551 CGCAAACTCT	2561 CGGTGTATAG	2571 GACGCTCAGC	AACGATCAAG	
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AATATAAAA	A GCGGGGAATG	GCTTGACCCC	GCGCAGAATG	2541 TCGATCTCTT	2551 CGCANACTCT	2561 CGGTGTATAG	2571 GACGCTCAGC	AACGATCAAG	
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AATATAAAA	A GCGGGGAATG	GCTTGACCCC	GCGCAGAATG	2541 TCGATCTCTT	2551 CGCAAACTCT	2561 CGGTGTATAG	2571 GACGCTCAGC	AACGATCAAG	- G
AATATAAAA	A GCGGGGAATG	GCTTGACCCC	2531 GCGCAGAATG	2541 TCGATCTCTT	2551 CGCAAACTCT	2561 CGGTGTATAG	2571 GACGCTCAGC	AACGATCAAG	
AATATAAAA	A GCGGGGAATG	GCTTGACCCC	2531 GCGCAGAATG	2541 TCGATCTCTT	2551 CGCANACTCT	2561 CGGTGTATAG	2571 GACGCTCAGC	AACGATCAAG	
AATATAAAA	A GCGGGGAATG	GCTTGACCCC	GCGCAGAATG	2541 TCGATCTCTT	2551 CGCAAACTCT	2561 CGGTGTATAG	2571 GACGCTCAGC	AACGATCAAG	- G

Figure 2. A multiple alignments of the 2031 OR nucleic acid sequence from A. fumigatus (SEQ 1,2) along with related 2031 ORs from other fungi and bacteria (see also Example 4). Regions 1-11, marked with * or #, refer to regions conserved at the amino acid level between Ors but not OYEs.

Fungal 2031 ORs are given by SEQ ID No.: SEQ ID Nos. 1, 2, 4, 5, and 7, A. fumigatus; SEQ ID No. 9, A. nidulans; SEQ ID Nos. 11 and 13, C. albicans; SEQ ID Nos. 15, 17 and 18, N. crassa; SEQ ID Nos. 20, 21 and 43, M. grisea; SEQ ID No. 23 (NP_595868), S. pombe; SEQ ID Nos. 25 and 26, C. trifolii; SEQ ID Nos. 28, 29, 31, 32 and 34, F. sporotrichioides; SEQ ID Nos. 36, 37 and 82, F. graminearum; SEQ ID Nos. 39 and 41, M. graminicola; SEQ ID No. 84, U. maydis.



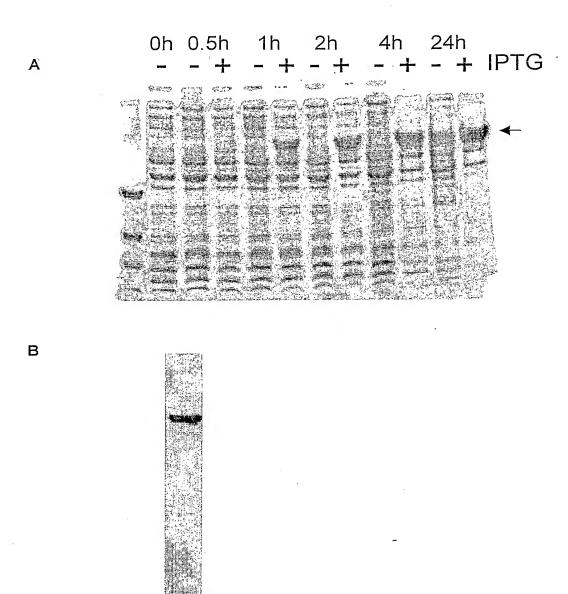


Figure 3. Recombinant 2031 OR. (A) Time course of recombinant 2031 OR induction over 24 hours after the addition of IPTG (samples without IPTG are also shown). The gel was stained with coomassie; A prominent band of the correct molecular weight (marked with an arrow) is seen. (B) Coomassie stained gel showing purified recombinant 2031.

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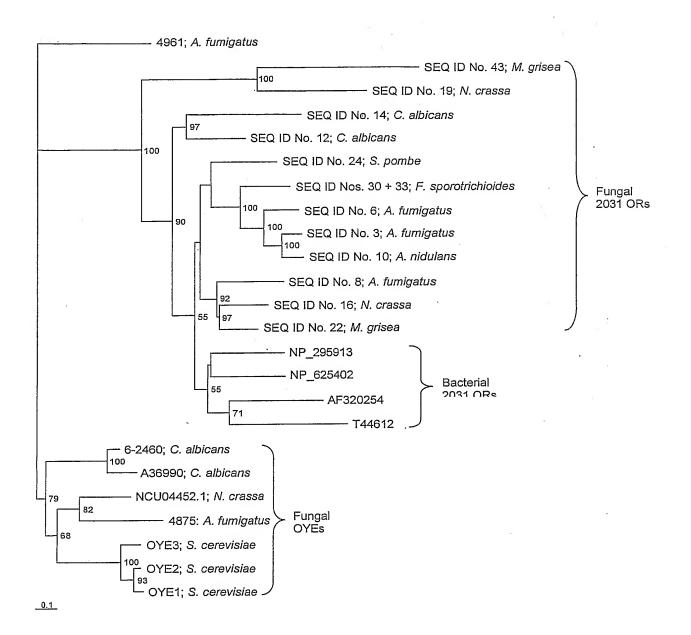


Figure 4. Phylogenetic tree showing relationships between *A. fumigatus* 2031 OR and similar proteins. This demonstrates a 2031 OR clade, which can be distinguished from the OYE proteins.

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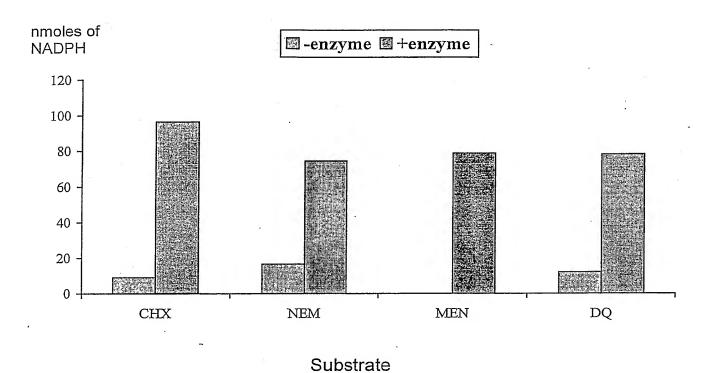


Figure 5: NADPH dehydrogenase activity of recombinant 2031 OR with cyclohexenone (CHX), N-ethylmaleimide (NEM), menadione (MEN) or duroquinone (DQ) as substrates. Final concentrations in the assay were as follows: 500 μ M substrate, 120 μ M NADPH, 1 μ g/200 μ L 2031 OR.

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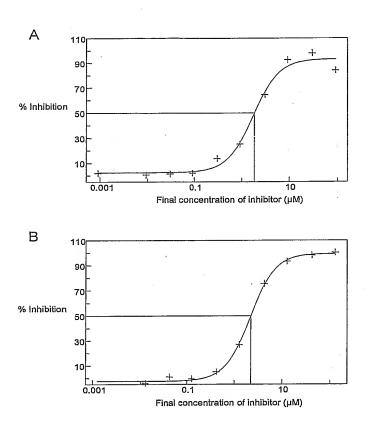


Figure 6: Inhibition of 2031 OR function by two inhibitors (shown in A and B) identified by high-throughput screening.

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